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NUTRITIVE VALUE OF HIGH AND LOW CALCIUM-CARRYING WHEAT†

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AN EXTENSIVE study of the mineral content of Utah-grown grains has revealed a wide variation in their calcium, magnesium, potassium, iron, sulfur, and chlorine content. The quantity of these elements in wheat varies with the variety and with the soil on which the wheat was grown, and further whether it has been grown under irrigated or dry-farm conditions. The average calcium content of the Utah-grown wheats was found to be about twice that reported for the 20-year averages for the Rothamsted wheats and considerably higher than the results reported by Sherman. The discovery of these differences is responsible for the following statement made by Greaves and Hirst (9):

> It is most probable that this (the high calcium content of the grains) would have a profound influence in the nutrition of farm animals which may be kept on a ration consisting primarily of grains. . . . This greater proportion of calcium to phosphorus in the Utah-grown grains should make of them a more nearly balanced food than is the case with the grains proportionately higher in phosphorus.

The present work is an attempt to evaluate, by animal-feeding tests, the importance of this difference.

Two varieties of wheat, Kota and Turkey, which vary widely in mineral content, were chosen for the work. The wheats were grown on the Nephi Dry-farm Substation on the same soil and under similar conditions. The analyses of the two wheats are given in Table I.

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TABLE I

PERCENTAGES OF PROTEIN AND MINERALS IN KOTA AND TURKEY WHEATS

Variety of wheat	Protein	Ca	Mg	P	K	Fe	S
Kota	17.41	0.080	0.182	0.308	0.334	0.006	0.253
Turkey	14.42	0.055	0.159	0.153	0.276	0.006	0.202

Each constituent, with the exception of iron, is higher in the Kota than in the Turkey wheat.

EXPERIMENTAL METHODS

Young albino rats, 22 to 25 days old, were placed in individual cages on experimental diets. Fresh water and food were given daily. Except for those placed in a dark room, the rats were kept in a well-lighted and ventilated room. Since wheat is low in protein, the wheat diets were supplemented with a basal diet consisting of: Casein, 24 grams; yeast, 32 grams; sodium chloride, 12 grams; and dried cabbage, 17 grams. This addition was expected to supplement the wheat, making a complete diet except for

TABLE II

GIVING DIETS, TOGETHER WITH SUPPLEMENTS AND CONDITIONS UNDER WHICH ANIMALS WERE KEPT

Group	Diet	Additions	Light
1	Wheat, 9 Basal, 1	None	Ordinary diffused
2	Wheat, 9 Basal, 1	None	None
3	Irradiated wheat, 9 Basal, 1	None	Ordinary diffused
4	Wheat, 9 Basal, 1	2 drops cod liver oil (daily)	Ordinary diffused
5	Wheat, 9 Basal, 1	Calcium carbonate	Ordinary diffused
6	Wheat, 9 Basal, 1	2 drops cod liver oil (daily) Calcium carbonate	Ordinary diffused

vitamin D and the varying amounts of calcium and phosphorus content of the wheat.

The conditions of feeding are summarized in Table II.

In Groups 5 and 6 of both Kota and Turkey wheats, the amount of calcium carbonate added to the diet was that quantity necessary to make the calcium-phosphorus ratio of the diet 2 to 1. As the calcium and phosphorus contents of the wheats were different, the amount of calcium carbonate added was different in each case.

The animals were kept on the diet for a period of three weeks. They were then anaesthetized, the blood drawn from the heart, pooled, and analyzed for calcium according to the Clark and Collip method (4). The blood phosphorus was determined according to the Fiske and Subbarow method (7). The tibia was dissected and used for the line test as outlined by McCollum and Simmons (12). The femurs were extracted with ether and alcohol, dried and ashed (2). The percentage of bone ash was calculated on dry weight after extracting the fat, as the fat content of the bones in animals is variable. On the ash of the femurs calcium determinations were made by the Tisdall and Kramer method (17); phosphorus determinations were made according to the Fiske and Subbarow method (7).

The wheats of Group 3 (Kota and Turkey) were irradiated for 30 minutes, 1 foot distant from the Hanovia quartz mercury vapor lamp, then mixed with the basal diet and fed to the animals.

Discussion of Results

Cowgill and co-workers (5) report results in which young rats were fed on rations where cereals furnished 62 to 93 per cent of the total calories. When the cereal content of the diet consisted of the smaller quantities the growth was exceptionally satisfactory, while in the diet containing sufficient cereal to yield 93 per cent of the calories, growth was not so satisfactory. In their experiments the cereal diets were supplemented with meat residue, salt mixture, cod liver oil, and 15 grams of fresh lettuce. In the experiment reported here the quantity of protein was not so great; neither was it of such a high biological value. Therefore, as rapid a growth as Cowgill reports could hardly be expected, although Cowgill gives no actual growth curves for making comparisons.

The growth curves for the different groups are given in Figures 1 to 6.

A close correlation exists in the growth of the animals on Turkey and Kota wheats when compared within the same groups, the only possible exception being in Group 5 where calcium carbonate supplements the two

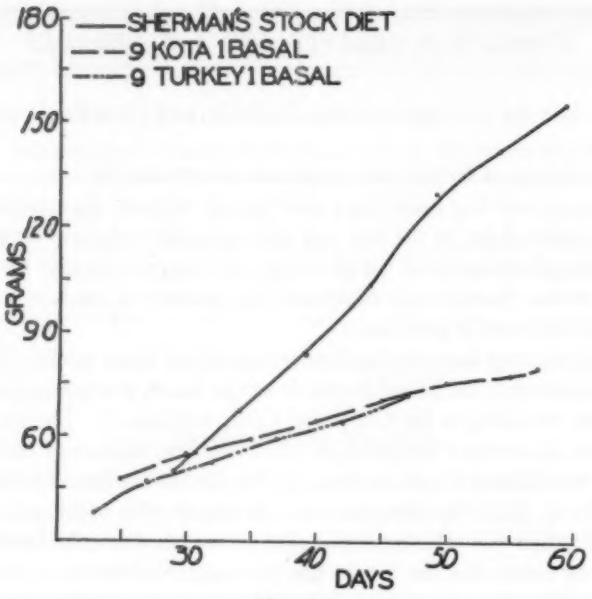
SHERMAN'S STOCK DIET
9 KOTA 1 BASAL
9 TURKEY 1 BASAL
180
150
120
90
60
GRAMS
30
40
50
60
DAYS

FIG. 1.

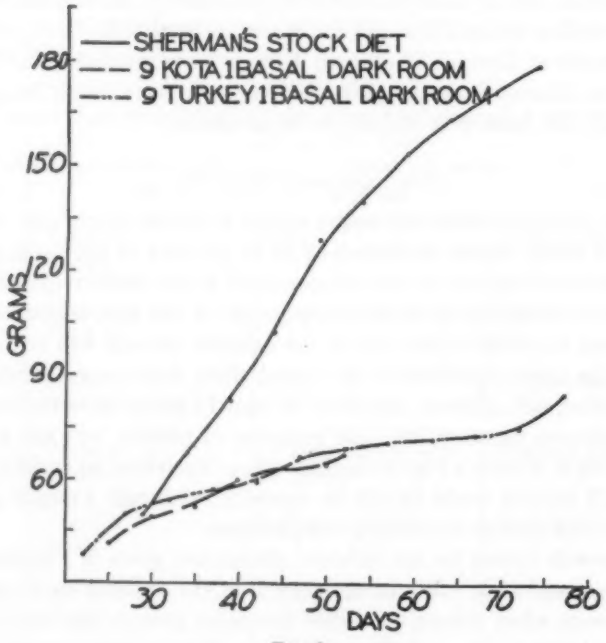
SHERMAN'S STOCK DIET
9 KOTA 1 BASAL DARK ROOM
9 TURKEY 1 BASAL DARK ROOM
180
150
120
90
60
GRAMS
30
40
50
60
70
80
DAYS

FIG. 2.

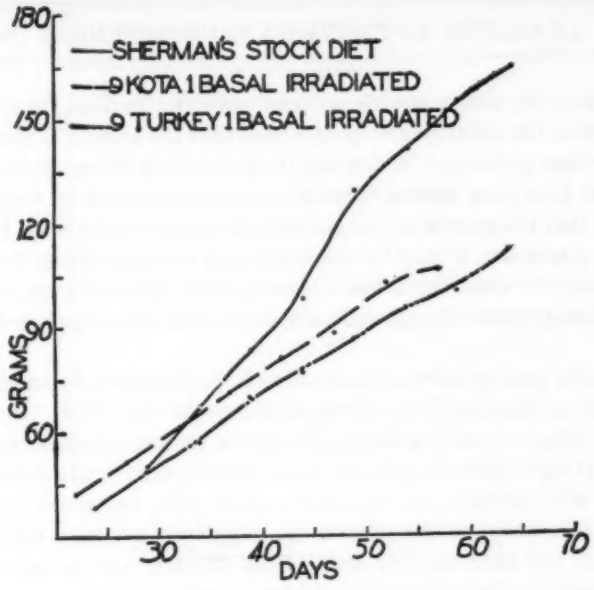
180
150
120
90
60
GRAMS
SHERMAN'S STOCK DIET
9 KOTA 1 BASAL IRRADIATED
9 TURKEY 1 BASAL IRRADIATED
30
40
50
60
70
DAYS

FIG. 3.

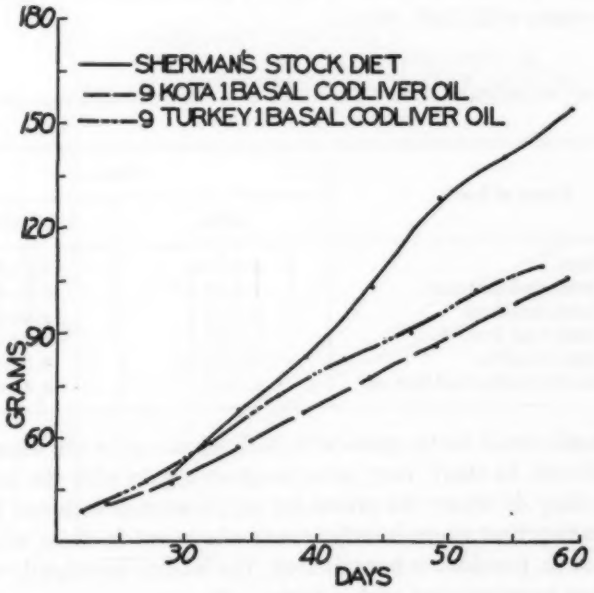
180
150
120
90
60
GRAMS
SHERMAN'S STOCK DIET
9 KOTA 1 BASAL CODLIVER OIL
9 TURKEY 1 BASAL CODLIVER OIL
30
40
50
60
DAYS

FIG. 4.

wheats. Here the better growth occurred with the Turkey. On comparing the growth in the different groups it is seen that the growth at similar ages is greater than in Group 1, indicating the presence of the mother substance of vitamin D in both wheats. This was activated to such an extent by irradiation that the growth of the animals was greatly improved. If growth is used as a measure, it may be concluded that no more activation of vitamin D occurred when the animals were kept in lighted rooms, where the sunlight had to pass through ordinary glass, than when kept in darkened rooms.

An equally good growth in these animals was obtained by irradiation of the wheat, as when cod liver oil was added to the diet. Thus, it is evident that these wheats contain a fair amount of the mother substance of vitamin D. In all groups, however, growth is not equal to that of the growth curve obtained with animals on Sherman's stock diet, but more nearly approaches this curve as the wheats are supplemented with calcium carbonate and cod liver oil. The diets of the different groups are probably deficient quantitatively in protein content as well as in the nature of amino acids making up the protein content.

The quantity of food required to make 1 gram of gain in weight is practically the same with both wheats.

TABLE III

GRAMS OF FOOD REQUIRED TO MAKE 1 GRAM GAIN IN WEIGHT (AVERAGE RESULTS OBTAINED ON FROM 7 TO 35 ANIMALS)

Grams of food	Wheat	
	Kota	Turkey
9 wheat+1 basal	6.5±0.3	6.2±0.4
9 wheat+1 basal, darkened room	7.5±1.3	7.6±0.5
9 wheat+1 basal, irradiated	4.3±0.1	4.6±0.1
9 wheat+1 basal+cod liver oil	4.5±0.2	4.9±0.1
9 wheat+1 basal+$CaCO_3$	4.7±0.3	4.0±0.1
9 wheat+1 basal+$CaCO_3$+cod liver oil	4.2±0.1	4.4±0.1

Animals make much better gains with both wheats after the wheats have been irradiated. In short, they make as great a gain with the irradiated wheats as they do where the grains are supplemented with cod liver oil, thus indicating that there is sufficient of the sterol in these wheats for normal growth, provided it be activated. The wheats are slightly more effective when supplemented with calcium carbonate.

Dutcher, Creighton, and Rothrock (6) established standards for differ-

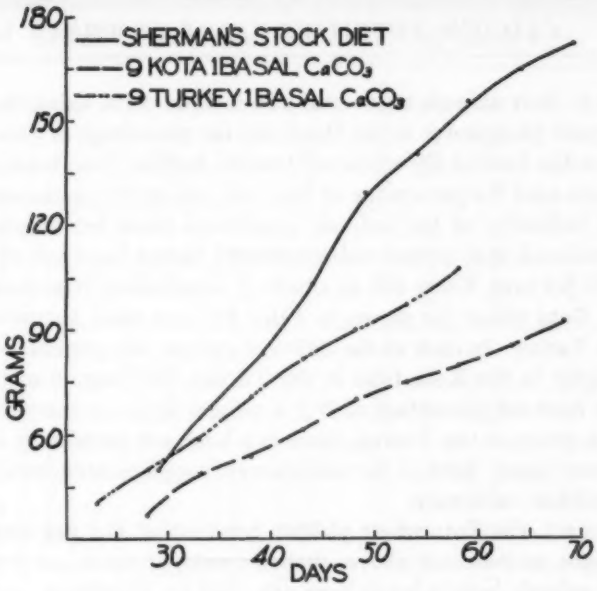
180
150
120
90
60
GRAMS
SHERMAN'S STOCK DIET
9 KOTA 1 BASAL CaCO3
9 TURKEY 1 BASAL CaCO3
30
40
50
60
70
DAYS

FIG. 5.

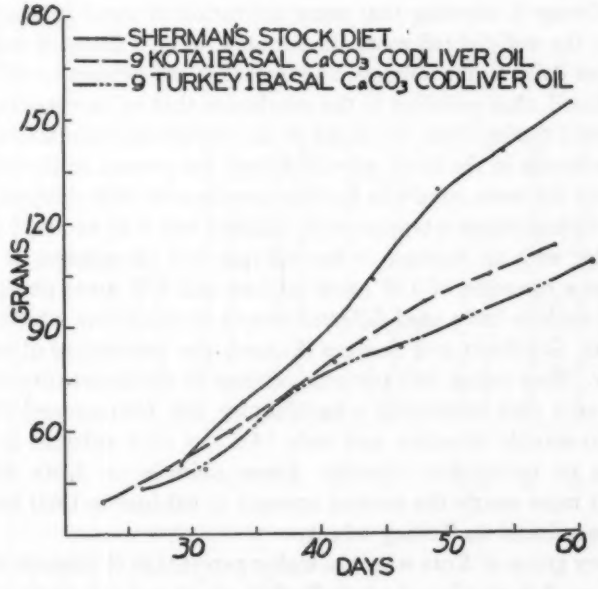
180
150
120
90
60
GRAMS
SHERMAN'S STOCK DIET
9 KOTA 1 BASAL CaCO3 CODLIVER OIL
9 TURKEY 1 BASAL CaCO3 CODLIVER OIL
30
40
50
60
DAYS

FIG. 6.

ent ages in their animals on normal and rachitic diets, using the level of the inorganic phosphorus in the blood and the percentage of bone ash calculated on the basis of dry-extracted femurs. Bethke, Steenbock, and Nelson (2) also used the percentage of bone ash, calculated on the same basis, as being indicative of the animals' conditions when fed different diets. They concluded that normal animals should have a bone ash of approximately 50 per cent. Using this as a basis of comparison, it seems apparent that the Kota wheat (as shown in Table IV) is a more nutritious wheat than the Turkey. In each of the different groups, the percentage of bone ash is higher in the Kota than in the Turkey. In Group 6 of the Kota, there is a bone ash percentage of 49.2, a normal figure; in comparison with this same group of the Turkey, there is a bone ash percentage of 32.4, a much lower figure. Both of the wheats were supplemented with cod liver oil and calcium carbonate.

In Group I, the Kota wheat yields a bone ash of 36.9 per cent. This is a low figure, as indicated above; these animals, however, are young, and younger animals have a lower bone ash. Bethke, Steenbock, and Nelson found a bone ash of 59 per cent in animals 66 days old and weighing from 200 to 275 grams. In both wheats, the bone ash of Group 2 is lower than that of Group 1, showing that some activation of sterol in animals' diet occurs in the well-lighted room. In Group 4, when 2 drops of cod liver oil were given daily to the animals, an increase in the percentage of bone ash was obtained, thus pointing to the conclusion that an increase in vitamin D over that coming from the sterol in the wheats will increase the deposition of minerals in the bone, provided these are present in the diet. Shohl (16) found the same results in feeding experiments with children. His calcium and phosphorus retention with children was 0.44 and 0.26 gram, respectively; with an increase in the calcium and phosphorus of the diet, there was a retention of 0.87 gram calcium and 0.49 gram phosphorus.

Other authors have used different means to determine whether a bone is normal. Goldblatt and Soames (8) used the percentage of calcium in the bone. They found 20.6 per cent calcium in the bones (dry weight) of animals on a diet containing a satisfactory salt mixture and furnishing ample fat-soluble vitamins and only 14.6 per cent calcium in animals receiving no fat-soluble vitamins. Those animals on Kota wheat approached more nearly the normal amount of calcium in their bones than did those animals on Turkey wheat.

In every group of Kota wheat, a higher percentage of calcium was found in the bone than was found where Turkey wheat was fed, again indicating a more nutritious wheat.

TABLE IV

COMPOSITION OF BONES AND BLOOD OF RATS REARED ON NINE PARTS WHEAT TO ONE PART BASAL DIET UNDER VARYING CONDITIONS

Group	No. Rats	Diet	Composition	Percentage			Blood	
				Bone Ash	Ca	P	Ca	P
1	15	9 wheat; 1 basal	Kota	36.9±0.5	13.4±0.2	6.8±0.1	6.7	14.5
	10	9 wheat; 1 basal	Turkey	29.4±0.6	10.0±0.2	5.0±0.1	6.5	10.0±0.1
2	10	9 wheat; 1 basal, animals in dark	Kota	34.8±0.8	12.4±0.2	6.4±0.1	6.1	6.7
	10	9 wheat; 1 basal, animals in dark	Turkey	25.7±0.4	9.0±0.1	4.5±0.1	6.0	6.0
3	34	9 wheat; 1 basal, wheat irradiated	Kota	32.4±0.4	12.4±0.2	6.7±0.1	6.2±0.1	11.2±0.2
	36	9 wheat; 1 basal, wheat irradiated	Turkey	30.3±0.4	10.9±0.2	5.8±0.1	6.7±0.1	11.6±0.1
4	14	9 wheat; 1 basal; 2 drops daily cod liver oil	Kota	36.4±0.8	14.6±0.3	7.6±0.2	12.2	17.0±0.8
	38	9 wheat; 1 basal; 2 drops daily cod liver oil	Turkey	31.5±0.3	11.5±0.1	6.0±0.1	6.9±0.3	10.8±0.2
5	10	9 wheat; 1 basal; $CaCO_3$	Kota	48.4±0.9	17.8±0.4	9.4±0.4	12.2	11.4
	10	9 wheat; 1 basal; $CaCO_3$	Turkey	34.6±0.3	13.3±0.1	6.6±0.1	5.6±0.2	8.3±0.1
6	10	9 wheat; 1 basal; 2 drops cod liver oil; $CaCO_3$	Kota	49.2±0.6	17.5±0.2	8.7±0.1	10.3	17.7
	20	9 wheat; 1 basal; 2 drops cod liver oil; $CaCO_3$	Turkey	37.4±0.6	14.0±0.2	7.2±0.1	9.4±0.4	8.9±0.1

The phosphorus content of the bones was invariably higher where the animals received Kota than where they received Turkey wheat; consequently, it is evident that although these are both dry-farm wheats and were raised on the same soil, Kota wheat under all conditions builds superior bones to the Turkey wheat.

Turning to a consideration of the calcium and phosphorus content of the blood, Cavins (3) reports the normal inorganic phosphorus content of the blood serum of rats as 7 to 8.5 mg. per 100 cc. serum. In animals rendered rachitic by a diet low in phosphorus (such as McCollum's Diet No. 3143), phosphorus is found to be about 3 mg. per 100 cc. and may run as low as 2 mg.

Dutcher, Creighton, and Rothrock (6) found the normal inorganic phosphorus of the blood serum to be 10 mg. per 100 cc. for rats 3 weeks old and 8 mg. per 100 cc. in 11-week old rats. If the rats were put on a rachitic diet (No. 3965, Steenbock) at 3 weeks of age, by the time they were 6 weeks old (21 days on the experimental diet) 1.6 mg. inorganic phosphorus per 100 cc. were found in the blood serum.

The blood calcium in Groups 1 and 2 of Kota wheat is fairly low, with an extremely high inorganic phosphorus for Group 1. However, these are not impossibly high figures, as Hess, Berliner, and Weinstock (10) report the inorganic phosphorus higher than our figures. The calcium content of the wheat is high, and the phosphorus content of the wheats increases proportionately. The blood calcium in Groups 1 and 2 of the Turkey wheat is low with a lower inorganic phosphorus than in the corresponding groups of the Kota wheat. As the serum calcium increases, the serum phosphorus decreases.

The symptoms of some of the animals in Group 2 on the Turkey wheat when kept in the dark room, together with an examination of the blood, indicated tetany.

The addition of cod liver oil or calcium carbonate, either separately or together, increased the calcium content of the blood of the animals fed on Kota wheat, but it required the addition of both to the Turkey wheat diet in order to obtain a normal calcium content of the blood. The phosphorus content of the blood of all animals, with one exception, was higher where Kota was fed than where Turkey was fed.

The line test was made on the tibiae of all animals. In Group 5, on the Kota wheat, the bones showed a higher percentage of calcium and phosphorus, although they were softer than those in Groups 4 and 6. The trabeculae of the animals in Group 5 were numerous and filled with black deposits of calcium. The zone of calcification appeared to be normal. In

comparing these bones with those of Group 4 in which cod liver oil had been added to the diet, a consistent difference was apparent. The zone of calcification in the latter group was normal, calcium deposits in the trabeculae being mainly absent. The bones were extremely hard and brittle and difficult to cut.

It is evident that there is a difference in the nutritive value of the two wheats, as is evidenced by both the bone and blood composition from the wheat. Moreover, even the addition of calcium carbonate and cod liver oil to the Turkey wheat did not make it the equal of Kota wheat as mirrored by the bone and blood results. This would lead one to believe either that the native calcium of the Kota is utilized better than the added calcium carbonate, or that there is a deficiency of phosphorus in the diet which is supplied in a measure by the extra phosphorus carried by the Kota wheat.

In some countries of the Orient, where cereals furnish as much as 84 per cent of the calories of the diet, it is likely that the calcium intake of the people is low. In this country, cereals, with the exception of milk, are the first foods usually given to children; consequently, the use of cereals carrying a high calcium content, together with cod liver oil, may change these border-line cereal diets to safe and economical ones.

The desirability of an ample supply of calcium in the diet during growth is important, as has been pointed out by Sherman and Booker (15). They state that although rats may show normal growth on limited calcium intake, they may still not have the optimum amount of calcium in their body. This is fully borne out by our results. An optimum amount of calcium in the body is accompanied by earlier maturity and extends the period between maturity and senescence. It is generally recognized that during the period of early childhood there is a great demand on the part of the body for calcium, in the formation of the teeth and bony tissue.

The importance of a high phosphorus content in the diet has not been given much consideration. However, Klein and McCollum (11) find dental caries in the teeth of rats whose blood phosphorus is below 10 mg. per 100 grams of serum. Wherever the blood phosphorus is above this critical figure, the rats appear to be immune to dental caries. If this is true, Kota wheat is a still more valuable wheat due to its high phosphorus as well as to its high calcium content. The blood figure is dependent upon the level of phosphorus, calcium, and vitamins ingested in the diet. The importance of having a high vitamin content in the diet for the prevention of dental caries was also pointed out by Mellanby (18). The blood phosphorus is higher for the animals fed on Kota wheat than for those fed on Turkey,

and it was only in the case of the animals fed on Turkey wheat that the phosphorus fell below the critical figure set by Klein and McCollum (11).

In the case of adults as well as children who eat large quantities of bread, there would be an advantage in the use of flours containing a higher percentage of calcium. According to Sherman (14) and Appleton (1), the average American diet does not contain enough calcium for maintenance and especially for optimum growth. The use of high calcium bread flour, therefore, cannot be looked upon as anything other than desirable. Therefore, this problem raises three important questions: 1.—What proportion of the calcium and phosphorus of these wheats would occur in the flour? 2.—Is there a sufficient difference to render the nutritive value of the flour of one wheat greater than the other? 3.—Is it possible to breed wheats of a high calcium and phosphorus content suitable for bread flour and which can be economically produced?

Summary

1. High- and low-calcium containing wheats were fed to rats, the wheat furnishing 90 per cent of the diet.

2. The percentage of bone ash, of calcium, and of phosphorus in the extracted bones, and the serum calcium and phosphorus were higher in all animals fed Kota (high-calcium) wheat than in those fed Turkey (low-calcium) wheat.

3. The advisability of using high-calcium wheats for both human and animal diets is indicated.

4. The question is raised as to what proportion of the calcium carried by the wheat occurs in the flour and whether it is feasible to breed high calcium-carrying wheats.

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EFFECT OF DIET ON EGG COMPOSITION

I. PARTIAL CHEMICAL ANALYSES OF EGGS PRODUCED BY PULLETS ON DIFFERENT DIETS

By

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THE egg of the domestic fowl being a common article of our dietary and a valuable food for convalescents and invalids, it is a matter of considerable importance to learn in what ways, and to what extent, the feed consumed by chickens may affect the composition and dietetic value of their eggs.

The diet of laying pullets and hens has an appreciable influence on the number and size of their eggs (Atwood, 1; Parkhurst, 2; Byerly, Titus, and Ellis, 3). The taste and odor of eggs and the color of their yolks may be modified by certain feeding stuffs. Onions, rape, turnips, and fresh fish, if fed in excess, impart strong flavors to the egg (Lippincott, 4). The principal natural pigment of egg yolk is xanthophyll, and by controlling the diet it is possible to markedly increase or decrease the amount of color (Palmer, 5). Capsanthin, the pigment of pimiento peppers, readily passes into the yolk (Morgan and Woodruff, 6). Certain synthetic coloring materials, such as Sudan III, when ingested by the chicken, may also pass into the egg yolk. Many fresh green feeding stuffs, such as grass, clover, and alfalfa have a tendency to cause the yolks to become more deeply colored.

Certain chemical compounds having no particularly distinctive color or odor may be found in the egg, if ingested with the usual feed. When benzoic acid is fed to chickens, it may be found later in their eggs (Carter, Howe, and Mason, 7). The percentage of iodine in the egg may be appreciably increased by feeding iodine compounds (Scharrer and Schrop, 8; Straub, 9; Jaschik and Kieselbach, 10). There is some evidence that the iron content of eggs may be increased by feeding certain feeds rich in iron (Hartung, 11; Hoffmann, 12) but on the other hand it has not been found

possible to increase it by feeding an iron supplement containing as much as 50 mg. of iron daily (Elvehjem, Kemmerer, Hart, and Halpin, 13).

The vitamin content of eggs also may be influenced by the feed consumed. By feeding chickens diets rich in vitamin A, eggs may be produced which are unusually rich in this factor (Bethke, Kennard, and Sassaman, 14). In a later paper on the effect of diet on egg composition data will be presented to show that the vitamin B content of eggs may be greatly influenced by diet. On the other hand the writers have observed little, if any, difference in the vitamin G content. By irradiating hens one may cause the vitamin D content of their eggs to be increased (Hughes, Titus, and Moore, 15; Hart and coworkers, 16).

Although it has been found that the vitamin content of eggs may be influenced by diet and that certain chemical compounds, some having distinctive colors and tastes, may be made to occur in eggs by ingesting them with the feed, there is but little evidence to show that the percentage of crude protein and ether extract may be influenced by diet. Working with pigeons, Pollard and Carr (17) and Gerber and Carr (18) have obtained evidence that diet may affect the composition of the eggs of this species. It is the purpose of this paper to present evidence that the composition of chicken eggs may be influenced by diet.

Experimental material

Rhode Island Red pullets of known pedigree were used. They were selected from a large number of pullets the ancestors of which had been bred for high egg production and uniformity of egg size for at least 7 generations. In making the selection a number about 50 per cent greater than that finally placed on experiment was taken. After eliminating the heaviest and lightest pullets the remaining ones were distributed among the several pens on the basis of live weight. This distribution was so made that the average weight of the pullets in any one pen differed from that of the pullets in any other of the pens by less than 50 grams and the standard deviation of the live weights was essentially the same for all pens.

The pullets were housed in a series of pens in two long, concrete-floored laying houses and were allowed the freedom of concrete-floored run-yards. The diets studied were fed from September 3, 1929, to August 12, 1930. At intervals of four weeks, beginning October 2, 1929, all eggs laid during a single day by the pullets in each of the pens were saved and analyzed. At the beginning of the experiment there were 40 pullets, each, on diets 2, 5, 8, and 9, and 25 pullets, each, on diets 10, 11, and 12.

THE DIETS

Most of the diets were compounded as follows:

	Parts by weight
Basal feed mixture	75
Yellow corn meal	500
Wheat bran	245
Rolled oats	150
Alfalfa leaf meal	55
Mineral mixture	5
Ground limestone	50
Steamed bone meal	30
Anhydrous sodium sulphate	10
Common salt	10
Special supplement	20
Cod-liver oil	2

The special supplements in the case of four of the diets, for which data are here presented, were:

Diet 2: Crab meal (the dried and ground offal of the crab fishing industry);

Diet 5: "Yeast Foam" (a dried yeast preparation sold for animal feeding purposes);

Diet 8: A mixture of 8 parts of desiccated meat meal (about 72 per cent protein), 7 parts of North Atlantic fish meal (dried and ground white fish offal), and 5 parts of dried buttermilk;

Diet 9: No special supplement, an additional 20 parts of the basal feed mixture were used.

The other three diets were made up as follows:

Diet No.	Pearled hominy and desiccated meat meal*	Mineral mixture**	Yeast†	Autoclaved yeast	Cod-liver oil
	Parts by weight	Parts by weight	Parts by weight	Parts by weight	Parts by weight
10	95	5	0	0	2
11	80	5	15	0	2
12	80	5	0	15	2

* In such proportions that the diet contained 20 per cent of crude protein before the addition of the cod-liver oil.

** The same as that used in diets 2, 5, 8, and 9.

† The same as that used in diet 5.

Several chemical analyses were made of each diet during the course of the investigation. The average results are given in the following table.

AVERAGE RESULTS OF PARTIAL CHEMICAL ANALYSES OF THE DIETS

Diet No.	Moisture	Crude ash	Crude protein	Ether extract	Crude fiber
	Per cent	Per cent	Per cent	Per cent	Per cent
2	11.25	11.23	15.64	5.33	5.80
5	11.41	6.23	11.21	6.57	4.13
8	11.45	6.56	20.62	6.27	3.52
9	10.84	5.77	11.53	6.10	5.00
10	9.94	6.97	19.95	5.14	1.05
11	10.08	6.48	19.47	5.09	1.31
12	9.56	6.48	20.01	5.23	1.31

The Average Composition of the Eggs

The yolks were separated from the whites as carefully as possible. All the eggs laid during a single day by the pullets in each pen were used in preparing the samples. Most of the samples, for which data are here given, contained not less than five eggs and in several cases the samples were derived from as many as twenty-two eggs. The essential analytical data are presented in Tables I, II, III, IV, and V.

TABLE I

AVERAGE DRY MATTER CONTENT OF THE YOLKS OF THE EGGS PRODUCED BY THE PULLETS ON THE SEVERAL DIETS

Date eggs were laid	Diet 2		Diet 5		Diet 8		Diet 9		Diet 10		Diet 11		Diet 12	
	Grams	Per cent	Grams	Per cent	Grams	Per cent	Grams	Per cent	Grams	Per cent	Grams	Per cent	Grams	Per cent
1929														
Oct. 2	6.05	51.2	6.66	52.0	6.28	50.3	5.54	51.1	—	—	—	—	—	—
Oct. 30	7.58	52.2	7.24	52.1	7.37	51.6	7.58	50.8	—	—	7.44	49.9	—	—
Nov. 27	6.86	49.1	7.77	55.9	8.10	52.9	7.56	52.3	7.61	51.9	7.94	50.7	8.02	52.2
Dec. 25	7.52	48.4	7.48	48.6	7.63	48.9	6.94	47.4	—	—	7.54	47.5	—	—
1930														
Jan. 22	9.37	55.6	8.74	55.3	9.02	53.1	8.24	53.8	—	—	8.35	52.9	—	—
Feb. 19	—	—	—	—	9.68	55.3	—	—	—	—	—	—	9.63	54.4
Mar. 19	8.95	51.5	8.97	52.0	9.18	52.1	9.27	52.9	8.97	53.4	9.06	53.3	8.58	52.3
Apr. 16	8.75	51.1	9.07	52.5	9.22	53.6	8.98	52.5	8.13	52.0	8.96	52.3	8.42	51.5
May 14	9.08	52.4	8.38	51.8	9.28	52.7	8.13	51.6	9.46	52.7	9.13	53.0	8.98	51.8
June 11	—	—	—	—	—	—	8.25	52.3	7.95	46.0	7.17	48.3	8.41	51.5
July 9	—	—	—	—	9.33	53.4	8.76	52.5	—	—	7.95	52.9	9.40	54.3
Mean	8.02 ±0.308	51.4 ±0.0565	8.04 ±0.216	52.5 ±0.582	8.51 ±0.253	52.4 ±0.407	7.93 ±0.248	51.7 ±0.396	8.42 ±0.267	51.2 ±1.034	8.17 ±0.179	51.2 ±0.529	8.78 ±0.162	52.6 ±0.350
σ*	1.29 ±0.218	2.37 ±0.400	0.91 ±0.153	2.44 ±0.412	1.18 ±0.179	1.91 ±0.287	1.16 ±0.175	1.85 ±0.280	0.89 ±0.189	3.43 ±0.731	0.79 ±0.126	2.35 ±0.374	0.64 ±0.115	1.37 ±0.248
C of V	16.11 ±2.786	4.61 ±0.779	11.27 ±1.924	4.65 ±0.786	13.92 ±2.140	3.64 ±0.550	14.64 ±2.255	3.59 ±0.396	10.51 ±2.298	6.70 ±1.435	9.70 ±1.557	4.59 ±0.731	7.26 ±1.315	2.61 ±0.471

* $\sigma = \sqrt{\frac{\sum(x-\bar{x})^2}{n-2}}$; this formula was used because of the small number of cases involved.

TABLE II

AVERAGE PROTEIN CONTENT OF THE DRY MATTER OF THE YOLKS OF THE EGGS

Date eggs were laid	Diet 2	Diet 5	Diet 8	Diet 9	Diet 10	Diet 11	Diet 12
	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent
Oct. 2, 1929	33.6	31.8	33.1	30.9	—	—	—
Oct. 30, 1929	33.5	32.4	32.3	31.6	—	—	—
Nov. 27, 1929	32.7	32.2	31.2	31.7	—	—	—
Dec. 25, 1929	32.8	32.1	32.4	32.2	—	32.4	—
Jan. 22, 1930	31.6	30.8	32.0	31.7	—	32.5	—
Feb. 19, 1930	—	—	31.9	—	—	—	31.7
Mar. 19, 1930	31.8	30.8	31.7	30.6	31.2	31.0	31.2
Apr. 16, 1930	32.7	30.7	31.6	30.3	31.4	31.9	31.3
May 14, 1930	32.3	31.3	31.3	32.1	32.1	31.6	—
June 11, 1930	—	—	—	31.7	30.0	31.2	32.3
July 9, 1930	—	—	31.4	31.5	30.0	30.3	31.3
Mean	32.6 ±0.18	31.5 ±0.18	31.9 ±0.13	31.4 ±0.14	30.9 ±0.32	31.6 ±0.22	31.6 ±0.16
σ*	0.77 ±0.130	0.75 ±0.128	0.62 ±0.094	0.67 ±0.100	1.06 ±0.227	0.87 ±0.156	0.52 ±0.112
C of V	2.37 ±0.400	2.39 ±0.403	1.95 ±0.294	2.12 ±0.320	3.44 ±0.735	2.74 ±0.494	1.66 ±0.354

TABLE III

AVERAGE FAT (ETHER EXTRACT) CONTENT OF THE DRY MATTER OF THE YOLKS OF THE EGGS

Date eggs were laid	Diet 2	Diet 5	Diet 8	Diet 9	Diet 10	Diet 11	Diet 12
	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent
Oct. 2, 1929	60.6	64.3	61.2	62.0	—	—	—
Oct. 30, 1929	58.6	59.1	60.0	60.8	—	59.6	—
Nov. 27, 1929	55.2	58.1	58.5	59.6	59.4	59.9	62.3
Dec. 25, 1929	—	—	56.3	55.7	—	52.3	—
Jan. 22, 1930	—	—	57.3	56.3	—	55.0	—
Feb. 19, 1930	—	—	61.9	—	—	—	56.9
Mar. 19, 1930	—	—	59.3	60.0	58.5	55.1	60.1
Apr. 16, 1930	—	—	61.7	61.8	59.1	58.6	59.0
May 14, 1930	—	—	59.5	56.3	54.7	57.2	54.7
June 11, 1930	—	—	—	—	57.0	55.9	57.6
July 9, 1930	—	—	61.3	60.9	60.0	57.4	60.0
Mean	58.1 ±1.50	60.5 ±1.83	59.7 ±0.43	59.3 ±0.60	58.1 ±0.60	56.8 ±0.59	58.7 ±0.70
σ*	3.86 ±1.603	4.71 ±1.296	2.02 ±0.305	2.67 ±0.424	2.19 ±0.427	2.63 ±0.418	2.73 ±0.492
C of V	6.64 ±1.836	7.78 ±2.155	3.39 ±0.512	4.50 ±0.717	3.77 ±0.735	4.63 ±0.738	4.65 ±0.840

* $\sigma = \sqrt{\frac{\sum(x-\bar{x})^2}{n-2}}$; this formula was used because of the small number of cases involved.

TABLE IV

AVERAGE DRY MATTER CONTENT OF THE WHITES OF THE EGGS PRODUCED BY THE PULLETS ON THE SEVERAL DIETS

Date eggs were laid	Diet 2		Diet 5		Diet 8		Diet 9		Diet 10		Diet 11		Diet 12	
	Grams	Per cent	Grams	Per cent	Grams	Per cent	Grams	Per cent	Grams	Per cent	Grams	Per cent	Grams	Per cent
1929														
Oct. 2	3.33	12.0	3.84	12.6	3.54	12.7	3.42	12.6	—	—	—	—	—	—
Oct. 30	3.49	12.6	3.38	12.9	3.98	13.3	3.74	13.1	—	—	4.09	13.5	—	—
Nov. 27	3.39	12.2	3.84	12.7	3.66	13.1	3.53	12.7	3.54	11.8	3.56	12.4	3.79	12.3
Dec. 25	—	—	—	—	3.19	10.5	2.95	10.8	—	—	3.26	10.2	—	—
1930														
Jan. 22	—	—	—	—	3.69	12.0	3.58	12.1	—	—	4.18	11.7	—	—
Feb. 19	—	—	—	—	4.24	13.1	4.13	12.5	—	—	3.86	11.9	3.34	11.7
Mar. 19	—	—	—	—	4.44	13.2	4.00	12.4	4.37	12.7	3.96	12.2	4.34	12.6
Apr. 16	—	—	—	—	3.95	12.1	4.05	11.8	3.88	11.6	3.65	10.7	3.94	12.7
May 14	—	—	—	—	3.75	11.9	3.42	11.5	3.88	13.1	3.84	11.6	4.25	12.4
June 11	—	—	—	—	4.22	12.2	3.84	12.0	—	—	3.89	12.3	3.84	10.9
July 9	—	—	—	—	4.13	12.2	4.19	12.7	—	—	3.23	10.9	3.16	10.6
Mean	3.40 ±0.045	12.2 ±0.168	3.69 ±0.146	12.7 ±0.084	3.89 ±0.078	12.4 ±0.174	3.71 ±0.081	12.2 ±0.140	3.92 ±0.141	12.3 ±0.296	3.75 ±0.073	11.7 ±0.217	3.81 ±0.121	11.9 ±0.236
σ*	0.11 ±0.031	0.43 ±0.119	0.38 ±0.103	0.22 ±0.060	0.38 ±0.055	0.86 ±0.123	0.40 ±0.057	0.69 ±0.098	0.42 ±0.100	0.88 ±0.029	0.34 ±0.052	1.02 ±0.153	0.48 ±0.086	0.92 ±0.167
C of V	3.36 ±0.926	3.52 ±0.970	10.19 ±2.835	1.70 ±0.468	9.87 ±1.433	6.96 ±1.066	10.69 ±1.555	5.64 ±0.814	10.68 ±2.576	7.13 ±1.733	9.14 ±1.390	8.66 ±1.316	12.51 ±2.290	7.77 ±1.409

* $\sigma = \sqrt{\frac{\sum(x-\bar{x})^2}{n-2}}$; this formula was used because of the small number of cases involved.

TABLE V

AVERAGE PROTEIN CONTENT OF THE DRY MATTER OF THE WHITES OF THE EGGS

Date eggs were laid	Diet 2	Diet 5	Diet 8	Diet 9	Diet 10	Diet 11	Diet 12
	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent
Oct. 2, 1929	87.2	87.8	88.2	—	—	—	—
Oct. 30, 1929	88.8	88.6	87.9	88.1	—	88.9	—
Nov. 27, 1929	90.5	89.7	89.2	88.9	90.1	90.2	89.7
Dec. 25, 1929	—	—	88.3	87.4	—	87.1	—
Jan. 22, 1930	—	—	87.8	87.8	—	87.4	—
Feb. 19, 1930	—	—	88.4	88.0	—	88.7	88.7
Mar. 19, 1930	—	—	90.0	87.9	87.4	88.9	88.4
Apr. 16, 1930	—	—	88.4	87.0	87.2	87.7	88.4
May 14, 1930	—	—	87.1	88.1	89.6	89.1	88.9
June 11, 1930	—	—	88.2	87.8	87.8	88.4	88.8
July 9, 1930	—	—	87.7	88.4	—	88.7	88.2
Mean	88.8 ±0.91	88.7 ±0.53	88.3 ±0.16	87.9 ±0.12	88.4 ±0.47	88.5 ±0.21	88.7 ±0.14
σ*	2.34 ±0.644	1.35 ±0.372	0.81 ±0.117	0.55 ±0.083	1.54 ±0.329	0.97 ±0.146	0.54 ±0.098
C of V	2.63 ±0.725	1.53 ±0.421	0.92 ±0.132	0.62 ±0.094	1.74 ±0.371	1.09 ±0.164	0.61 ±0.110

* $\sigma = \sqrt{\frac{\sum(x-\bar{x})}{n-2}}$; this formula was used because of the small number of cases involved.

DISCUSSION

Inasmuch as the pullets to be used in this study were to be fed in groups, the experimental work was planned and carried out in such a way as to avoid, as far as possible, the effect of individuality. To this end special attention was given 1.—to the homogeneity of the "population" from which the experimental birds were selected, 2.—to the manner of distributing the pullets among the several groups, and 3.—to the size of the groups and the method of obtaining the egg samples from the several groups.

The pullets were taken from an unusually homogeneous "population."[12] As previously stated, their ancestors had been bred for high egg production and uniformity of egg size for at least seven generations. To insure homogeneity in live weight, as well as in breeding, all heavy and all light in-

dividuals were discarded before the pullets were distributed among the several pens.

The distribution of the pullets among the pens was so made that, at the start, the average live weight of the pullets in any one pen was within 50 grams of the average live weight of those in any other pen and the standard deviation of the live weights was essentially the same for all pens.

A sufficiently large number was placed in each pen to insure obtaining enough eggs for a suitable analytical sample on any one day after the pullets began to lay. The collection of eggs for these samples was made at regular intervals of four weeks and the number of eggs included in most of the samples varied from five to twenty-two. That the samples thus taken were random, in so far as the group from which they were obtained is concerned, is indicated by the fact that no single pullet contributed to more than 7 of the 11 samples from any one pen and, on the average, less than one-fourth of the pullets contributed to more than 3 samples.

Thus it is evident that the several groups were as similar as it was possible to make them and that any individuality which may have been originally inherent in any group could not have exhibited itself uniformly, or even approximately so, in all eleven samples. It now remains to ascertain whether, or not, the differences found were due to chance alone or to the diets.

The usual method of comparing means and their probable errors shows that there were no statistically significant differences between the eggs laid by the pullets receiving the several diets, in the case of:

(a) the weight of dry matter in the yolks,
(b) the percentage of dry matter in the yolks,
(c) the percentage of fat in the dry matter of the yolks,
(d) the weight of dry matter in the whites,
(e) the percentage of dry matter in the whites, and
(f) the percentage of protein in the dry matter of the whites.

However, this method does show that the eggs laid by the pullets on diet 2 (the diet which contained 20 parts of crab meal) had a significantly higher percentage of protein in the dry matter of their yolks than did the eggs from the pullets on diets 5, 9, 10, 11, and 12, the differences being from 3.6 to 5.2 times their probable errors. Had there been a sufficiently large number of samples on which fat determinations had been made, it is very probable that here, too, differences could be demonstrated.

Although the usual method of comparing means and their probable errors is applicable, when it is desired to show differences in average composition, it is not wholly suitable in the present case because of the rela-

tively small number of analyses. For the present purpose "Student's" method is the more appropriate one to use. This method is applicable when a number of paired values are to be compared. In this investigation the

TABLE VI

TABULATION OF THE COMPARISONS MADE BY "STUDENT'S" METHOD

Comparison	The chance of obtaining a difference equal to, or greater than, the one observed, if, a priori, no difference in the composition of the eggs is to be expected
Weight of dry matter in the yolks	
8> 2	1 in 10
8> 5	1 in 16
8> 9	1 in 213**
8>10	1 in 5
8>11	1 in 25
8>12	1 in 21
Percentage of protein in the dry matter of the yolks	
2> 5	1 in 3500***
2> 8	1 in 100**
2> 9	1 in 178**
8>12	1 in 38
11> 5	1 in 29
Weight of dry matter in the whites	
5> 2	1 in 7
8> 2	1 in 32
9> 2	1 in 26
8> 9	1 in 308**
Percentage of protein in the dry matter in the whites	
11> 9	1 in 199**

Comparison	The chance of obtaining a difference equal to, or greater than, the one observed, if, a priori, no difference in the composition of the eggs is to be expected
Percentage of dry matter in the yolks	
5> 9	1 in 28
10> 2	1 in 29
Percentage of fat in the dry matter of the yolks	
5> 2	1 in 35
8> 2	1 in 27
9> 2	1 in 55
8>11	1 in 184**
9>10	1 in 81*
9>11	1 in 79*
Percentage of dry matter in the whites	
5> 2	1 in 76*
8> 2	1 in 233**
9> 2	1 in 370**
8>11	1 in 240**

* These values are on the borderline of significance.
** These values are significant.
*** This value is distinctly significant.

composite samples of eggs collected during a single day once every four weeks yielded a number of series of paired analytical data.

All possible comparisons were made and those which revealed statistically significant differences, and a few which did not, are tabulated in Table VI. The rows of the tabulation are read as follows (as examples, (a) the third row under "weight of dry matter in the yolks," and (b) the first row under "percentage of protein in the dry matter in the yolks"): (a) "The eggs resulting from diet 8 had more dry matter in their yolks than those from diet 9. There is only one chance in 213 that this or a greater difference could have occurred due to chance alone." (b) "The eggs resulting from diet 2 had a greater percentage of protein in the dry matter of the yolks than those from diet 5. There is only one chance in approximately 3500 that this or a greater difference could have occurred due to chance alone."

A priori, one might expect to find that eggs produced by pullets receiving a well balanced diet (diet 8) would be different in composition from those resulting from a diet derived almost exclusively from plant sources (diet 9), or from a diet of very simple composition (diet 11). Such appears to be the case, for the eggs resulting from diet 8 contained more dry matter in the yolks than those from diet 9. They also contained a higher percentage of fat in the dry matter of the yolks than those from diet 11; and more dry matter in the whites than those from diet 9. A study of Table VI shows that the eggs laid by the pullets receiving diet 8 differed in several ways from the eggs resulting from the other diets.

The effect of diet 2 on the composition of the eggs is noteworthy, in view of the fact that this diet contained only a single source of animal protein, i.e., crab meal. The eggs resulting from feeding this diet had a greater percentage of protein but a smaller percentage of fat in the dry matter of the yolks than those from diets 5, 8, and 9; they also had a smaller percentage of dry matter in the whites.

The eggs resulting from the feeding of the diet derived chiefly from plant sources (diet 9) contained more fat in the dry matter of the yolks than those from diets of much simpler composition (diets 10 and 11), but they contained less protein in the dry matter of the whites.

In general, diet seemed to have less effect on the composition of the whites than it did on that of the yolks.

It is obvious from the comparisons tabulated in Table VI that differences in the composition of eggs were brought about by feeding different diets to the pullets producing the eggs. That such differences may be caused by diet is noteworthy. In the second paper of this series a sensitive

biological method will be described by means of which it is possible to demonstrate other differences.

Summary

Composite samples of eggs laid on the same days by pullets of the same age and breeding but receiving different diets were analyzed, and by means of a suitable statistical method it was shown that the composition of these eggs was affected by diet.

The eggs produced by pullets receiving a well balanced diet differed in composition from eggs resulting from the feeding of less satisfactory diets.

The yolks appeared to be more readily affected by diet than the whites.

The most consistent difference, although it was small, was observed between the percentage of protein in the dry matter of the yolks of eggs laid by pullets receiving a diet containing crab meal and those receiving the other diets. This difference was demonstrable by the usual method of comparing means and their probable errors, as well as by "Student's" method.

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THE EFFECT OF DAIRY MANUFACTURING PROCESSES UPON THE NUTRITIVE VALUE OF MILK

II. THE APPARENT DIGESTIBILITY OF FRESH WHOLE MILK AND OF POWDERED WHOLE MILK

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GROWTH experiments in this laboratory in which albino rats were fed fresh whole milk, evaporated milk, and powdered whole milk raised a question concerning the effect of manufacturing processes upon the digestibility of milk. Among the factors involved in these processes which may possibly affect digestibility are heating, homogenization, concentration by evaporation of water, and drying. Heating is involved in most of the manufacturing processes.

Many studies of the effect of heat upon the chemical compounds of milk have been made. Before the development of modern methods of pasteurization, comparisons of cooked and sterilized milk with raw milk were a popular subject of study. Later many investigations of the effect of pasteurization were conducted. These experiments consisted largely of studies of changes in chemical composition and changes effected in digestibility as determined by means of artificial digestive fluids and by employment of animals with stomach fistulas or of human subjects from whom the stomach contents were removed by means of stomach pumps. Experiments of this sort undoubtedly yield valuable information, but they do not necessarily present a complete or reliable picture of the normal digestive process in its entirety. In the normal process of digestion, the food is first subjected to enzymic action in an acid medium. Later the resulting digestive products are acted on by a different set of enzymes in a neutral medium. During these processes the end-products are removed. It is difficult to reproduce these conditions in experiments conducted in vitro. Food residues which are recovered from the stomach give but a partial picture of the complete, normal process. There is no certainty that experiments conducted in vitro will yield results comparable with the complete life process. The variation between the results of the extensive experiments of Wallen-Lawrence and

Koch (23) with evaporated and raw milk carried out in vitro and the results recently reported by the authors (Nevens and Shaw, 14) of experiments with animals is an illustration of this lack of agreement.

So far as the authors have been able to discover only a few determinations of the completeness of digestibility of the constituents of milk have have been made in experiments involving the normal, entire digestive process.

The earliest studies of the digestibility of cows' milk which have come to the authors' attention are those of Camerer (3), Prausnitz (15), Rubner (17), and Forster (5). Each of the first three of these investigators reports a three-day or four-day experiment upon a human subject receiving milk as the only food. Presumably the milk was raw except in Prausnitz' experiment, in which it was sterilized by heating for two hours in a steam sterilizer. The coefficients of apparent digestibility calculated from the data given by the three investigators, respectively, are as follows: total nitrogen, 94.3, 91.7, and 93.5; fat, 97.2, 94.9, and 95.1; dry matter, 92.9, 90.8, and 92.1. It will be observed that the coefficients for the sterilized milk are slightly lower than those for the raw milk. Both Rubner and Prausnitz state that protein and sugar could not be found in the feces. Forster found that a child failed to digest only 6.35 per cent of the dry substance of milk. The dry feces contained 34 per cent ash and 30 to 40 per cent fat.

Rubner (18) later conducted another experiment with a man weighing 70 kgm. during a two-day period in which the man consumed 2,500 cc. of milk daily, and also a one-day period in which he consumed 3,000 cc. He reports the undigested constituents of the milk for the two periods to be, in percentage, respectively: nitrogen, 7.02 and 12.91; fat, 2.57 and 7.12; dry matter, 5.72 and 11.16; sugar, 0 and 0.

Wasileff-Petersburg (25) conducted digestion experiments with six healthy young people 18 to 23 years of age. He found that the proteins of raw milk were more completely digested than those of cooked milk. A larger quantity of volatile fatty acids was found in the excreta after drinking cooked milk than after taking raw milk, leading to the conclusion that cooking lowers the digestibility of the fat.

Raudnitz (16) using a dog during three-day and four-day experimental periods found cooked milk slightly less digestible than raw milk.

Terroine and Spindler (22) employed pigs weighing 8 to 10 kgm. in their investigations of three methods of pasteurization. Their experiments showed no differences in the digestibility of the proteins or mineral constituents of the pasteurized and unpasteurized milk.

Several experiments have been carried out showing the effect of heat on

the efficiency of utilization of the components of milk. Washburn and Jones (24) and Magee and Harvey (10) found that the proteins of heated milk were less efficiently utilized by pigs than the proteins of raw milk.

Morgan (12) obtained similar results with rats in a comparison of raw casein and casein toasted at 150° to 200°C. for 30 to 45 minutes. The biological value was lowered although the digestibility was changed only a little.

Miyawaki, Kanazawa, and Kanda (11), using white rats as experimental animals, found coefficients of apparent digestibility of the proteins of four brands of dried milk ranging from 86 to 91. When the coefficients were obtained in artificial digestion experiments with pepsin, they ranged from 78 to 92, and 10 coefficients for raw milk had an average of 84.4.

The results of numerous experiments conducted in vitro lack uniformity. This lack of agreement may be explained in part by lack of uniformity in the intensity and duration of the heating process, in the condition of the milk before heating, and in the methods employed in the digestion processes. A few of the experiments are given brief mention.

Fleischman and Morgen (4) found that the longer milk was heated above 85°C. the less digestible the proteins became.

Baginsky (2) reports that in the case of completely sterilized milk the casein was less readily precipitated by rennin and the digestibility in gastric juice was lower than in the case of raw milk.

Somerville (19) in a series of 30 pancreatic digestion experiments found that the protein of milk reconstituted from powder was more completely digested than raw milk. These results coincided with his earlier results using pepsin.

Krull (9) reported the digestibility of the components of milk dried by the roller process to be: protein, 95.5 per cent; lactose, 99 per cent; fat, 97 per cent.

Stassano and Talarico (20) found that dry milk was more completely digested during tryptic digestion than raw milk.

Aviragnet, Dorlencourt, and Bloch-Michel (1) showed by experiments in vitro that milk dried by heating rapidly, at a high temperature, is more completely digested by trypsin than is raw milk.

Grimmer, Kurtenacher, and Berg (7) found that heating milk at 60°C. rendered the lactalbumen less soluble and less digestible.

Hess, Koch, and Sennewald (8) state that "boiling milk definitely reduces its soluble protein content but facilitates peptic digestion."

Gratz (6) reports no changes in digestibility of milk brought about by pasteurizing for 30 minutes.

Experimental Methods

The experiments reported in this paper deal only with studies of the completeness of digestibility of cows' milk. It is assumed that the differences between the amounts of protein, fat, sugar, and total solids ingested and the amounts of these appearing in the feces represent the amounts digested. In making this assumption, it is recognized that products of endogenous origin are present in the feces. Since this is an entirely normal and necessary part of the digestion process, it is reasonable to assume that the loss of these substances in the feces is one of the costs of digestion. Further, the extent of the loss is undoubtedly closely related to the amount and character of the particular food or mixture of foods ingested. It is believed, therefore, that the assumption that the digested nutrients are equivalent to ingested nutrients minus feces nutrients, which is the method of calculation commonly used in both human and animal nutrition, gives a highly reliable picture of the completeness of digestibility. The coefficients obtained by this method are often termed coefficients of "apparent digestibility."

Albino rats were used in these feeding tests. They were paired according to sex and approximate weights. The methods of feeding and management which were employed in previous experiments (Nevens and Shaw, 13, 14) were followed as far as possible. The rats were confined individually in glass cages below which pans lined with heavy filter paper were placed to receive the excreta. The fresh milk was fed in glass sponge dishes. For the powdered milk a modified McCollum feeding device was used. Practically none of the powder was spilled from these devices; in the few cases in which spilling did occur the milk was carefully collected and returned. The animals fed powdered milk were supplied with distilled water. Since it was impracticable to attempt to equate food intakes in this experiment, food was supplied *ad libitum*.

Fresh milk was fed twice daily. It was secured from the University Dairy Barns daily and each lot was sampled before feeding. Analyses were made of a composite sample. The milk used in Periods 1, 2, 4, and 5 consisted each day of the mid-portion of a single milking of an individual cow. The term "whole milk" as used in this and previous papers (Nevens and Shaw, 13, 14) refers to milk obtained in this manner. The milk supply for Periods 3 and 6 was secured from the mixed milk of four cows. The term "fresh" refers to unheated milk in excellent condition and not over 24 to 28 hours old when fed.

Two commercial brands of powdered milk were used, one being manufactured by the "spray" process and the other by the "roller" process.

A sufficient amount of powdered milk to supply each animal during the entire digestion trial was weighed into numbered glass jars. A sample for analysis was taken during the weighing. The powdered milk was placed in the feed dishes daily or often enough to keep a supply before the animals.

The digestion periods were each ten days in length. These trials were preceded by a preliminary period of three to five days, during which the particular experimental food to be tested was fed. Before each new digestion trial, stock mixture was fed for several days in order to keep the animals in good physical condition.

Feces were removed from the collection pans daily. In the protein digestion trials they were transferred directly to stoppered Kjeldahl flasks containing 50 cc. C.P. sulfuric acid. When it was evident that some of the feces had been contaminated by urine, they were transferred, before placing in the Kjeldahl flasks, to a sheet of clean filter paper laid over screen wire and washed by a stream of hot, slightly acidified, nitrogen-free water from a wash bottle. In the digestion trials for dry matter, ether extract, and sugar, the feces were transferred to paraffined paper cups, in which they were dried for two to three hours at a temperature of 60° to 70°C. They were then placed in glass jars provided with rubber rings and glass covers and kept in a refrigerator until analyzed.

The methods of analysis prescribed by the Association of Official Agricultural Chemists were followed. Qualitative tests only for sugar were made, both Benedict's and Fehling's methods being employed.

Discussion of Results

The apparent digestibility of the protein of fresh whole milk was found to be substantially higher than that of powdered whole milk. The mean of 47 coefficients previously reported (Nevens and Shaw, 14) is $92.3 \pm .17$, while the mean of 29 coefficients reported in this paper is $90.9 \pm .20$. The mean for the 76 determinations is $91.8 \pm .14$.

The range of the 26 coefficients of apparent digestibility of protein in powdered whole milk (Table I) is 84.4 to 90.9. The mean of the coefficients is $87.4 \pm .22$. But little difference was found in the coefficients for spray process milk and for roller process milk.

In a few cases the amounts of protein consumed individually by pair mates were widely different. It is believed, however, that a comparison of the records of pair mates is permissible, since the intake of a smaller or larger amount of protein than the average for the group seemed to have no influence upon digestion coefficients. For example, in the case of Pairs 301–302 and 303–304 (Table I, Period 2) the protein intake shows a rather

TABLE I

COMPARATIVE APPARENT DIGESTIBILITY OF THE TOTAL PROTEIN OF FRESH WHOLE MILK AND OF POWDERED WHOLE MILK

Animal No.	Live weight (grams)	Protein in milk consumed (grams)	Coefficient of apparent digestibility	Animal No.	Live weight (grams)	Protein in milk consumed (grams)	Coefficient of apparent digestibility
Period 1							
Powdered whole milk (spray process)				Fresh whole milk			
291	294	29.1	89.4	292	271	26.7	91.2
293	208	19.7	89.1	294	211	23.3	91.1
295	216	21.9	84.6	296	195	20.4	91.2
297	217	25.0	*	298	206	21.8	91.6
299	200	22.0	86.7	300	203	21.6	91.2
301	167	17.8	88.4	302	164	20.4	92.6
303	241	29.0	*	304	258	23.8	93.4
305	183	23.9	90.9	306	179	20.9	91.7
335	267	28.0	*	336	250	25.5	91.5
337	240	22.0	89.1	338	223	23.3	92.5
Period 2							
Fresh whole milk				Powdered whole milk (spray process)			
291	321	25.5	90.9	292	271	25.7	85.8
293	223	23.5	92.2	294	205	16.7	86.6
295	228	22.0	91.1	296	204	23.8	88.0
297	228	21.0	90.2	298	209	22.6	86.6
299	203	19.9	91.3	300	197	16.3	87.1
301	177	20.2	90.9	302	165	14.9	88.1
303	277	23.8	92.9	304	310	31.5	88.6
305	192	22.5	91.0	306	188	20.0	85.7
335	315	25.5	*	336	272	27.0	87.6
337	270	25.3	92.4	338	247	22.6	87.6
Period 3							
Fresh whole milk				Powdered whole milk (roller process)			
363	165	18.7	88.4	364	162	21.9	85.8
365	177	21.2	88.2	366	158	19.6	85.0
367	201	18.7	89.7	368	218	27.3	88.4
369	140	11.7	88.4	370	156	18.0	87.9
371	204	21.2	89.9	372	218	25.4	90.1
373	146	14.2	92.6	374	159	23.0	86.1
375	170	16.7	89.4	376	156	19.6	85.8
377	177	16.3	85.8	378	187	17.2	84.4
379	193	15.6	91.4	380	218	27.3	*
381	194	18.7	90.6	382	206	22.3	89.0

* Determination of protein in feces lost.

Note: The records of pair mates are shown on the same line. Thus Nos. 291 and 292 are pair mates, etc.

wide difference but the coefficients are much the same as those for the other animals of their groups.

The differences shown by pair mates in the apparent digestibility of protein indicate with a high degree of probability that there is a true difference in the nutritive qualities of the two kinds of milk. An average difference as great as that found with the seven pairs of animals fed fresh whole milk and powdered whole milk in Period 1 (Table I) might be expected to occur by chance but once in 470 trials conducted in the same manner. Likewise, an average difference as great as that found with the nine pairs of animals used in Period 2 (Table I) might be caused by chance not more than once in 10,000 repetitions of the experiment. For Period 3, the probability is once in 220 trials. These calculations of probabilities follow the method of Student (21).

The reversal trials (Table I, Periods 1 and 2) give further weight to the evidence that the apparent digestibility of the protein of fresh whole milk is higher than that of powdered whole milk. Not only is the coefficient secured with each animal fed fresh whole milk higher than that of its pair mate fed powdered whole milk, but the apparent digestibility of protein by each animal was more complete when fed fresh whole milk than when the same animal was fed powdered whole milk.

The authors (Nevens and Shaw, (14) previously reported that 45 coefficients of apparent digestibility of the protein of evaporated milk had a mean of $88.4 \pm .25$. The rather close agreement of that result with the result here given for powdered whole milk ($87.4 \pm .22$) is interesting in view of the fact that evaporation is usually one of the procedures in the process of drying milk.

The fat of both fresh whole milk and powdered whole milk was found to be highly digestible (Table II). The mean of 30 coefficients for fresh whole milk is $98.7 \pm .06$, while for an equal number of coefficients for powdered whole milk it is $98.7 \pm .08$. There were no significant differences in the apparent digestibility of the fat of the two brands of powdered milk.

In view of the likelihood that ether soluble substances other than fat are normally present in the feces, it seems reasonable to assume that the fat of milk is almost completely, if not entirely, digestible. It is often claimed that manufacturing processes, such as homogenization, which reduce the fat globules to smaller size, increase the digestibility of the fat. The results reported in this and the previous paper (Nevens and Shaw, (14) raise a question regarding the soundness of such claims, since the digestibility of the fat of fresh whole milk is so nearly complete that there is but little possibility of its being increased.

TABLE II

COMPARATIVE APPARENT DIGESTIBILITY OF THE FAT AND TOTAL SOLIDS OF FRESH WHOLE MILK AND OF POWDERED WHOLE MILK

Animal No.	Live weight grams	Fat in milk consumed grams	Total solids in milk consumed grams	Coefficient of apparent digestibility		Animal No.	Live weight grams	Fat in milk consumed grams	Total solids in milk consumed grams	Coefficient of apparent digestibility	
				Fat	T. solids					Fat	T. solids
Period 4											
Fresh whole milk						Powdered whole milk (spray process)					
291	346	21.0	84.3	99.7	92.1	292	299	22.4	76.6	99.2	90.7
293	233	19.2	77.3	99.5	92.2	294	210	19.5	66.9	99.7	91.3
295	241	19.7	79.2	99.6	93.7	296	210	12.2	41.9	99.5	89.1
297	240	18.3	73.6	99.2	92.5	298	223	18.7	64.0	99.5	89.6
299	217	17.6	70.8	99.0	92.7	300	203	19.2	65.7	99.5	90.9
301	184	13.7	55.0	99.5	92.7	302	170	16.1	55.1	99.5	92.1
303	305	19.2	77.3	98.0	92.4	304	358	33.7	115.4	99.2	91.5
305	205	16.6	66.9	99.4	92.7	306	203	20.1	68.9	99.5	91.1
335	348	24.4	98.2	98.8	92.7	336	303	25.9	88.7	99.3	92.1
337	303	19.3	77.4	98.8	91.4	338	280	23.3	79.6	99.2	91.0

Period 5

Fresh whole milk						Powdered whole milk (spray process)					
363	124	16.7	63.7	98.3	92.1	364	116	20.3	72.4	97.7	90.1
365	130	17.5	66.6	97.9	92.1	366	115	23.0	82.2	97.8	89.2
367	140	17.7	67.4	98.2	91.5	368	130	20.4	72.7	98.0	93.5
369	105	13.0	49.5	98.1	93.0	370	109	19.2	68.4	97.9	90.8
371	129	16.8	64.0	98.4	92.6	372	118	22.0	78.4	97.5	90.0
373	114	13.9	53.2	98.1	91.9	374	116	18.8	67.2	98.1	90.8
375	112	13.9	52.9	98.4	92.8	376	105	16.9	60.1	97.7	90.2
377	138	15.6	59.4	98.9	92.0	378	131	22.4	79.9	98.5	91.7
379	134	14.2	54.1	98.7	92.3	380	138	23.4	83.3	97.9	88.8
381	131	15.7	59.8	98.4	90.8	382	125	17.5	62.4	98.1	92.5

Period 6

Fresh whole milk						Powdered whole milk (roller process)					
363	178	22.6	89.8	98.9	91.6	364	172	24.2	84.7	99.0	91.1
365	190	22.9	91.1	98.5	91.0	366	173	23.5	82.1	98.8	91.2
367	214	19.1	75.9	98.6	91.2	368	245	26.1	91.3	99.1	92.5
369	157	15.3	60.9	98.1	91.8	370	164	19.7	69.0	98.7	91.0
371	223	21.6	85.9	98.7	91.8	372	253	29.7	103.7	98.3	89.4
373	162	18.3	72.8	98.0	90.9	374	176	26.2	91.7	99.3	91.3
375	195	19.9	79.1	98.7	93.1	376	172	25.4	88.8	98.7	91.0
377	199	21.2	84.3	98.9	90.9	378	196	19.6	68.7	99.1	91.8
379	222	21.3	84.8	98.4	92.0	380	238	29.9	104.6	98.8	90.3
381	211	21.5	85.5	99.0	90.6	382	240	27.3	95.5	98.8	90.8

Careful tests by both Benedict's and Fehling's methods have failed to reveal the presence of sugar in the feces of animals fed either fresh whole milk or powdered whole milk. Tests on individual collections of feces from 20 animals fed fresh whole milk, 10 animals fed spray process powdered whole milk, and 10 animals fed roller process powdered whole milk were all negative for sugar. It is believed, therefore, that the sugar of fresh whole milk and of powdered whole milk is completely digestible.

Assuming that the digestibility of the protein in the different kinds of milk varies, that the fat is more than 98 per cent digestible and that the sugar is completely digestible, then it is to be expected that the digestibility of the total solids of a particular sample of milk is influenced largely by the digestibility of its protein. This expectation is in harmony with the experimental results (Table II).

Thirty coefficients of apparent digestibility of the total solids of fresh whole milk have a mean of $92.0 \pm .09$, while the mean of an equal number of coefficients for powdered whole milk is $90.9 \pm .13$. It is evident that these coefficients are slightly higher in each case than those for protein of the respective kinds of milk, and that fresh whole milk is superior to powdered whole milk in digestibility of total solids. These coefficients for total solids lie between the coefficients for protein and for the other solids, but are not quite so high as would be expected by calculation. They tend, however, to substantiate the results found for protein, which indicate lower digestibility values for the powdered milk.

Many references are found in the literature to the "ease of digestibility" of certain kinds of milk, and the term "more digestible" is also commonly used. Many of these terms are deduced from the observations of physicians in cases in which they have found that one kind of milk agrees with the patient, while another kind causes more or less digestive disturbance or is unsatisfactory for some reason. There are many statements to the effect that evaporated milk and dried milk are "more digestible" than fresh raw milk.

The authors believe that their work helps to clarify the situation which now exists with respect to the term digestibility as applied to milk. Claims that homogenization, evaporation, or drying, or a combination of these factors, makes the protein and fat of milk more *completely* digestible lack the support of adequate experimental evidence obtained in actual feeding tests. The authors' findings, however, do not preclude the possibility that manufacturing processes such as those just mentioned may affect the time required for the digestion of the protein and fat, or that they may make the milk more readily tolerated by some individuals. In view of the differ-

ences between individuals with respect to their tolerance for certain foods, it is entirely possible that in some cases the *total* nutritive effect of evaporated milk and of powdered milk may be more favorable than that of fresh whole milk.

Summary and Conclusions

The apparent digestibilities of the total protein, fat, sugar, and total solids of fresh whole milk, spray process powdered whole milk, and roller process powdered whole milk were studied in feeding tests with albino rats. No significant differences in the apparent digestibility of the two kinds of powdered milk were found.

The apparent digestibility of the protein of fresh whole milk was significantly higher than that of powdered whole milk. It was shown in reversal trials that the higher coefficients for the fresh whole milk were not related to the individuality of the animals but that each animal digested the protein of fresh whole milk more completely than that of powdered whole milk.

The fat of both fresh whole milk and powdered whole milk was found to be about 99 per cent digestible, while the sugar of both kinds of milk was completely digestible.

Coefficients of apparent digestibility of total solids lay between those for protein and those for fat and were higher in the case of fresh whole milk. This substantiates to some extent the findings with respect to the apparent digestibility of protein.

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FACTORS WHICH DETERMINE RENAL WEIGHT

XIII. THE HEAT PRODUCTION OF THE RAT AS VARIED BY THYROID ADMINISTRATION

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THE administration of an active thyroid preparation has been shown (MacKay and MacKay, 7) to lead to an increase in the weight of the kidneys greater than the increase in protein intake incident to the increased food consumption could account for. Experiments have been performed to examine the relative influence of the active thyroid material upon the kidney weight and the heat production of the organism.

Desiccated thyroid[1] was used in this study. In the first experiment the diet described in Table I was used. Four cages of male albino rats were fed from 90 to 195 days of age. One group (B1) received no thyroid. The

TABLE I

CONTROL DIET

Commercial casein	20
Cornstarch	36
Dried powdered brewer's yeast (Harris)	10
Salt mixture (Osborne and Mendel)	4
Cod liver oil	10
Lard	20

other groups received (B2) 0.12 per cent, (B3) 0.18 per cent and (B4) 0.30 per cent respectively. At the end of the 105 days the rats were starved for 24 hours and their basal oxygen consumption determined under chloretone anesthesia using the method described by Dock (3). A tracheal cannula was closely connected to a 6 cm. glass tube containing soda lime for carbon dioxide absorption, which in turn led into a small bottle filled with strongly alkaline solution. A thermometer, the tube from the oxygen tank and the spirometer, a 20 cc. Luer syringe fitted with a kerosene lubricated Bakelite float, also led into this. The temperature of the system varied inappreciably during the required four or more measurements of the oxygen utilization rate. The environmental temperature was maintained at 29°C. The

[1] Wilson Laboratories, U. S. P.

rats were then sacrificed and their kidneys weighed with precautions described before (MacKay and MacKay, 5). The data obtained are presented in Table II. The administration of thyroid material naturally increased the caloric intake and hence the amount of protein ingested. This of itself would increase the weight of the kidneys (MacKay, MacKay, and Addis,

TABLE II

Per 100 sq. cm. body surface

No.	Group	Body weight gm.	Body surface sq. cm.	Kidney weight mgm.	Food intake gm./day	Protein intake gm./day	Kidney weight due to protein mgm.	Corrected kidney weight mgm.	Oxygen consumption cc./min.
1	B1	278	483	165	2.09	0.46	25	140	0.87
2	B1	274	478	168	2.09	0.46	25	143	0.79
3	B1	302	511	170	2.09	0.46	25	145	0.85
4	B1	282	488	177	2.09	0.46	25	152	0.86
5	B1	287	494	193	2.09	0.46	25	168	0.83
6	B2	246	445	209	3.17	0.69	38	171	1.33
7	B2	227	422	212	3.17	0.69	38	174	1.36
8	B2	230	426	231	3.17	0.69	38	193	1.31
9	B2	220	413	233	3.17	0.69	38	195	1.78
10	B3	167	344	253	3.78	0.82	45	208	1.74
11	B3	218	411	266	3.78	0.82	45	221	1.75
12	B3	212	404	282	3.78	0.82	45	237	1.88
13	B4	198	386	294	3.97	0.87	48	246	2.30
14	B4	193	379	277	3.97	0.87	48	229	2.06
15	B4	178	359	288	3.97	0.87	48	240	2.48

9). When the protein is casein, the relationship between the protein ingested and the kidney weight may be expressed (MacKay and MacKay, 6) as *protein intake* (grams per 100 sq. cm. body surface per day) $= 0.0183$ *renal weight* (milligrams per 100 sq. cm. body surface) $- 2.75$ or the kidney weight is dependent upon the protein as *renal weight* $=$ *protein intake* $\times 55$. Our results have all been corrected for the effect of ingested protein in order to make their comparison with the oxygen consumption more simple. As heretofore, all kidney weights are the average figure for the two kidneys. The kidney weight corrections made necessary by the fluctuation in the protein intake are very different in the four groups and another experiment was carried out with the same diet containing 0 (A1), 0.06 (A2), 0.12 (A3), 0.18 (A4) and 0.24 (A5) per cent desiccated thyroid respectively, identical in every way with the first except that 15 days before the

end of the experiment some of the casein in the various diets was replaced with cornstarch. The data for this group comprise Table III. The protein corrections are somewhat more uniform.

TABLE III

		Per 100 sq. cm. body surface							
No.	Group	Body weight gm.	Body surface sq. cm.	Kidney weight per 100 cm^2 mgm.	Food intake gm./day	Protein intake gm./day	Kidney weight due to protein mgm.	Corrected kidney weight mgm.	Oxygen consumption cc./min.
1	A1	286	492	160	1.77	0.43	24	136	0.83
2	A1	284	490	162	1.77	0.43	24	138	0.71
3	A1	308	518	175	1.77	0.43	24	151	0.85
4	A1	281	487	177	1.77	0.43	24	153	0.78
5	A1	288	495	192	1.77	0.43	24	168	0.89
6	A2	246	445	208	2.85	0.50	28	180	1.19
7	A2	270	473	210	2.85	0.50	28	182	1.27
8	A2	246	445	214	2.85	0.50	28	186	1.19
9	A2	230	426	215	2.85	0.50	28	187	1.22
10	A2	240	437	216	2.85	0.50	28	188	1.44
11	A3	250	450	191	2.98	0.48	27	164	1.62
12	A3	218	411	216	2.98	0.48	27	189	1.58
13	A3	195	382	225	2.98	0.48	27	198	1.93
14	A3	235	432	249	2.98	0.48	27	222	1.62
15	A3	210	402	254	2.98	0.48	27	227	1.82
16	A4	184	368	262	4.16	0.57	31	231	1.85
17	A4	188	373	273	4.16	0.57	31	242	2.17
18	A4	190	375	292	4.16	0.57	31	261	2.16
19	A5	207	397	260	4.53	0.62	34	226	1.94
20	A5	198	386	269	4.53	0.62	34	235	2.15
21	A5	204	394	291	4.53	0.62	34	257	2.30
22	A5	212	404	327	4.53	0.62	34	283	2.44

The data for both experiments have been plotted in Figure 1. Body surface was calculated by the formula devised by Carman and Mitchell (2). There is a definite, probably linear, relation between the kidney weight and the basal oxygen consumption of the organism. When we measure the total heat production of the organism by the food intake, a reasonable procedure since the animals were neither gaining nor losing weight, there is also a linear relationship (Fig. 2) between this figure and the kidney weight. This indicates that the thyroid material raised the total metabolism of our rats solely through its effect upon basal heat production.

The linear relation between the basal oxygen consumption of the organism and the kidney weight is very similar to that found for heart weight (Smith and MacKay, 11). In the case of heart weight it was possible to offer a reasonable explanation of the relationship upon the basis of the probable work performed by the organ. With the kidney this is not so simple. The excretion of the products of exogenous protein catabolism is

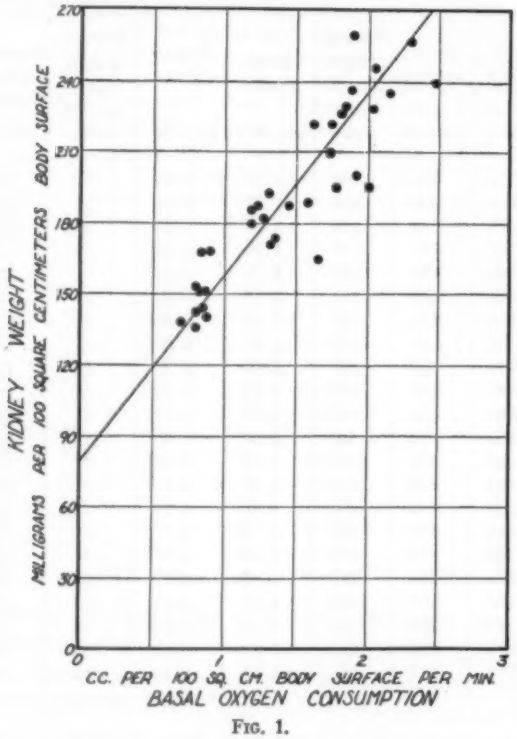

FIG. 1.

obviously part of the work of the kidney. The effect of this work on the kidney has been subtracted before considering the relation to the basal oxygen consumption in Figure 1. There is the possibility that the renal weight as varied by thyroid is governed by the endogenous protein catabolism which is increased (MacKay and Cockrill, 4) by thyroid. However an increase in the endogenous protein catabolism produced by starvation or dehydration is not followed by an increase in renal weight (MacKay and Cockrill, 4) and we are of the opinion that the relation between kidney

weight and the endogenous protein metabolism, when both are varied by thyroid, is not causal but due to their dependence upon the same factor, namely, the increased level of the basal metabolism.

Phosphate excretion is increased by thyroid (Aub, Bauer, Heath, and Ropes, 1) and an increased phosphate excretion due to feeding inorganic phosphates produces an increase in the weight of the kidneys (MacKay,

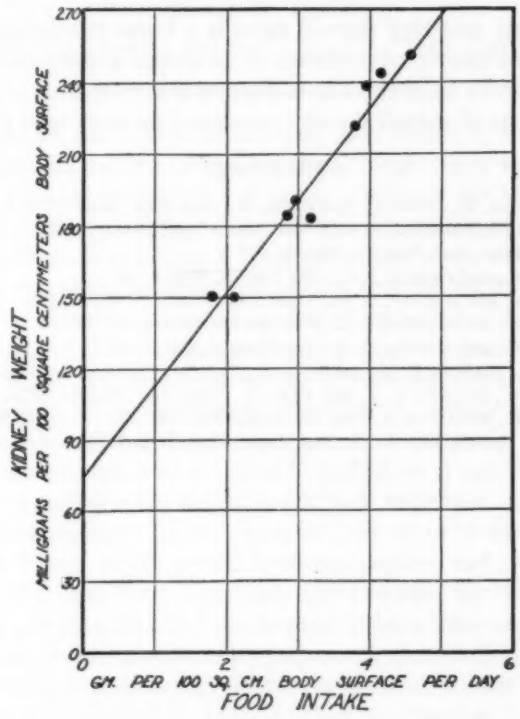

FIG. 2.

MacKay, and Addis, 8). It seems very unlikely that an increase in the urinary phosphate excretion contributes a significant part of the renal weight increase due to thyroid for the relation between renal weight and the basal metabolism is very good while between urinary phosphate excretion and the basal metabolism it is only roughly positive. Furthermore, kidneys of the weight found after thyroid administration, if due to phosphate, would show definite pathology (MacKay and Oliver, 10) and in the present experiments all of the kidneys were normal.

In the present state of our knowledge of the effect of thyroid upon the weight of the kidneys we are unable to offer any reasonable explanation for the increase in renal weight following thyroid administration and the relationship between the renal weight and the heat production of the organism when this is varied by thyroid.

Summary

In albino rats receiving thyroid there is a linear relationship between renal weight and caloric requirements of the animal as measured by oxygen consumption under basal conditions (fasting and chloretone anesthesia) or under conditions of normal activity (measured by daily food intake).

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FACTORS WHICH DETERMINE RENAL WEIGHT

XIV. THE RELATIVE INFLUENCE OF AMINO, UREA AND PROTEIN NITROGEN IN THE DIET

By

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IT has been shown (MacKay, MacKay, and Addis, 2) that urea nitrogen in the diet has less effect upon renal weight than a corresponding amount of nitrogen in the form of casein. Although the ultimate fate of most of the protein nitrogen is urea nitrogen, it actually reaches the body as amino nitrogen. The influence upon the weight of the kidneys of young male albino rats of amino, urea, and protein nitrogen has been compared.

The protein nitrogen was fed as casein and the amino nitrogen as glycine and glutamic acid. The cost of the dietary ingredients precluded any large number of rats in the diet groups but we believe that the great care which was exercised in all measurements compensates in large degree for the small number of animals. In other experiments on renal weight effect in young rats the animals were fed from 26 to 70 days of age. We have found that diets regulating renal weight reach their maximum effect within 10 days. In this experiment the rats were fed from 60 to 70 days of age. During the last 9 days of this period food consumption and the excretion of nitrogen in the urine were measured. The averages for this period form the basis of our data. In any experimental series a litter mate was placed upon each diet so that a series of a group on the control diets and one upon each of the other four diets represents several sets of 5 litter mates each.

The diets are described in Table I. They are identical in caloric value and, within a given group, in nitrogen content. The control (C) diet contained 3.75 per cent nitrogen, 2.73 per cent of which was in the form of casein. All of the "A" diets contained 2.38 per cent more nitrogen than the control diet or 6.13 per cent. The "B" diets contained twice as much additional nitrogen as the "A" diets giving a total of 8.52 per cent. The casein used was a specially purified product marketed by the Harris Laboratories (Tuckahoe, N. Y.). Powdered brewers yeast came from the same source. Osborne and Mendel's (5) salt mixture was used. The urea, glutamic acid, and glycine were recrystallized from C. P. products.

TABLE I
COMPOSITION OF DIETS

Diet	C	1A	2A	3A	4A	1B	2B	3B	4B
Casein	20.	37.5	20.	20.	20.	54.9	20.	20.	20.
Yeast	10.	10.	10.	10.	10.	10.	10.	10.	10.
Cod liver oil	10.	10.	10.	10.	10.	10.	10.	10.	10.
Salt mixt.	4.	4.	4.	4.	4.	4.	4.	4.	4.
Cornstarch	50.	32.5	44.8	25.	37.2	15.1	39.7		24.4
Lard	6.	6.	6.	6.	6.	6.	6.	6.	6.
Urea			5.2				10.3		
Glutamic acid				25.				50.	
Glycine					12.8				25.6

The rats upon all of the diets did reasonably well with the exception of those receiving the higher concentration of glutamic acid. The animals disliked this food and their intake was insufficient for maintenance. This does not however impair the value of our average figures. After ten days

TABLE II
ORGAN WEIGHTS AND RELATIONSHIPS

Diet	No. Rats in group	Gross Body Weight		Length mm.	Body Surface sq. cm.	Per 100 square centimeters per day				
		Initial gm.	Death gm.			Heart Weight mgm.	Food Intake per day gm.	Protein Intake per day gm.	Nitrogen Intake as protein** per day gm.	Nitrogen Excretion per day mgm.
C	3	112	165	188	331	187	3.78	0.737	0.790	70
1A	3	115	156	187	322	184	3.51	1.210	1.252	138
2A	3	114	154	186	323	185	2.64	0.515	0.943	185
3A	3	113	131	177	282	177	3.17	0.618	1.132	117
4A	3	114	152	183	309	188	3.55	0.693	1.267	179
C	3	105	140	179	292	197	3.36	0.656	0.720	90
1A	3	106	147	177	298	203	3.45	1.190	1.233	181
2A	3	106	142	176	292	198	4.02	0.784	1.438	219
3A	3	108	131	174	268	200	3.36	0.656	1.201	160
4A	3	109	132	175	273	206	3.34	0.652	1.215	195
C	4	122	160	183	321	173	3.48	0.680	0.731	86
1B	4	121	136	177	293	170	3.22	1.585	1.635	181
2B	4	121	153	182	305	172	3.56	0.695	1.810	261
3B	4	120	111	172	244	169	1.78	0.348	0.905	115
4B	4	120	133	176	271	170	2.68	0.524	1.362	190

** The yeast nitrogen not included as protein under the observed protein intake is excluded here.

on the diets the rats were starved for 12 hours and then killed. Anatomical measurements were made by methods described elsewhere (MacKay and MacKay, 1). The results are presented in Table II.

The dietary protein has a marked effect upon the renal weight. When this protein is casein this weight may be expressed (MacKay and MacKay, 3) as: *Renal Weight* (mgm. per 100 sq. cm. body surface) = (*Protein Intake* (gm. per 100 sq. cm. B. S.)+*2.75*) ÷ *.0183* or the renal weight due to protein may be expressed: *Renal Weight* = *Protein Intake* × *55*. These formulae were used for treating the data in Tables II and III. Since the kidney weight calculated from the actual protein intake (Table III) is somewhat higher for the high protein diets, a great deal higher for the urea diet and much less for the amino acid diets than the observed figures, we confirm our earlier result that urea has less effect on renal weight than protein nitrogen and conclude that amino nitrogen both in the form of glutamic acid and glycine has a definite influence upon renal weight. From the

TABLE II

ORGAN WEIGHTS AND RELATIONSHIPS

Per 100 square centimeters per day									
Kidney* Weight actual mgm.	K. Wt. calc. from protein intake mgm.	Difference per cent	K. Wt. calc. from N. intake as protein mgm.	Difference per cent	K. Wt. calc. from N. excre. as protein mgm.	Difference per cent	Heart: kidney ratio	Difference per cent	Per cent H_2O in Kidney
195	191	−2	193	−1	174	−11	1.05	100	75.4
200	216	8	218	9	197	−1	1.09	105	74.3
196	179	−9	202	3	214	9	1.05	101	74.6
186	184	−1	212	14	190	2	1.06	101	74.5
204	188	−8	219	2	212	4	1.08	103	74.0
193	186	−4	189	1	181	−6	1.02	100	76.4
223	215	−4	217	1	212	−5	1.10	108	76.4
206	193	−6	229	19	225	9	1.04	103	75.9
193	186	−4	216	16	205	6	0.97	95	74.7
219	186	−16	218		217	−1	1.06	104	75.6
178	186	4	190	6	180	1	1.03	100	76.6
209	237	12	240	15	212	1	1.22	117	76.3
193	188	−3	249	29	239	22	1.13	110	75.6
212	169	−23	200	−6	190	10	1.26	123	75.6
231	179	−22	224	−3	215	−7	1.36	132	75.9

* Average weight of the two kidneys as used throughout this study.

TABLE III

KIDNEY WEIGHT DEPENDENT ON PROTEIN INTAKE*
(Protein Intake in grams × 55 = Kidney Weight in Milligrams)

Diet	Observed**				Calculated from the actual protein intake				
	A1	A2	B	Average	A1	A2	B	Average	Per cent dev.
C	45	43	28	39	41	36	37	38	−3
1	50	73	59	61	60	60	80	66	+14
2	46	56	43	48	29	43	38	36	+28
3	36	43	62	47	34	36	19	29	−35
4	54	69	81	68	38	35	29	34	−47

Calculated from the Nitrogen intake as protein					Calculated from the Nitrogen excretion as ingested protein				
A1	A2	B	Average	Per cent dev.	A1	A2	B	Average	Per cent dev.†
43	39	40	40	+3	24	31	30	28	0
68	67	90	75	+23	42	62	62	55	+18
52	79	99	77	+106	63	75	90	76	+86
62	66	50	56	+19	40	55	40	44	+22
69	68	74	70	+3	62	67	65	64	+22

* Mgms. per 100 sq. cm. body surface.

** Determined as difference between 150 (required for zero protein intake) and actual figure for renal weight in mgms. per 100 sq. cm. body surface.

† Adjusted to zero basis for control group.

average results of the relation of the renal weight calculated from the nitrogen intake considered as protein, and particularly from the nitrogen excretion in the urine considered as food protein, to the observed figures, we may conclude that nitrogen in the form of glutamic acid and glycine have practically the same influence upon renal weight as casein nitrogen and that the nitrogen of all of these are more effective in increasing the weight of the kidneys than urea nitrogen. It is interesting that none of the diets had a significant effect upon the water content of the kidneys.

When the basal metabolic rate is raised by the administration of thyroid the weight of the kidneys is increased (MacKay, Smith and Closs, 4). The possibility existed that casein nitrogen had a greater influence on renal weight than urea nitrogen because casein raises the metabolic rate while

urea does not. Since glutamic acid, which is without influence upon the metabolic rate, and glycine which raises it, are similar in their effect upon renal weight, we must discard this hypothesis.

SUMMARY

Amino nitrogen in the form of glutamic acid and glycine have practically the same influence on renal weight as protein nitrogen in the form of casein. Urea nitrogen is less effective than amino or protein nitrogen.

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IS FLUORINE AN INDISPENSABLE ELEMENT IN THE DIET?*

By

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FLUORINE is the most active, chemically, of the elements. It is widely distributed in soils, rocks, and waters, being estimated to constitute 0.1 per cent of the first half mile of the earth's crust, land and sea. It is placed twentieth in order of abundance among the elements in the surface half mile of the earth. In certain spring and well waters it is so abundant as to cause mottling of the enamel of the teeth of persons drinking such waters while the enamel is forming (1). Fluorine is apparently universally present in plant and animal tissues. The question is still unanswered as to whether the element plays a physiological rôle or is present in the tissues as an accidental constituent because it is ingested in all foods.

Morichini (2) in 1802 first found fluorine in biological material when analyzing fossil elephant molars. Since that time many investigators have examined numerous animal and plant tissues for fluorine. Interest has centered mainly in the fluorine content of teeth and bones. Trebitsch (3) gives a review of the analyses up to 1927. The soft tissues are poor in fluorine, but bones, enamel, and dentine are relatively rich in it.

McCollum and co-workers (4) first described the effects of feeding to rats a diet containing 226 parts per million of sodium fluoride. This amount is excessive in comparison with the amounts generally consumed by humans or animals in food and water. Rats fed a diet otherwise satisfactory but containing excessive fluorine showed defective dentition. Christiani and Gautier (5) report that fluorine tends to accumulate in the bones of guinea pigs producing a condition resembling osteomalacia. Begara (6) reported that feeding excessive amounts of fluorine retarded calcification of the osseous tissues and produced dark bands in the incisors. Smith has extended these investigations to human subjects (1). The present paper is a report of experiments to determine whether satisfactory nutrition can be

* The data reported in this paper are taken from the dissertation of Dr. George R. Sharpless, submitted in partial fulfillment of the requirements for the degree of Doctor of Science in Hygiene.

secured during growth and reproduction with diets depleted as far as possible in fluorine.

Method of Determining Fluorine

The quantitative methods for the determination of fluorine were studied critically and a combination of the volitalization procedure of Reynolds, Ross and Jacobs (7) and of Wagner and Ross (8) and the colorimetric method of Merwin (9) and Stieger (10) was adopted. Our procedure was based upon the formation and volatilization of silicon tetrafluoride when a substance to be analyzed is intimately mixed with fine silica and decomposed with concentrated sulfuric acid. The colorimetric method was based upon the bleaching action of fluorine on the yellow color of an oxidized titanium solution.

Reagents

Concentrated H_2SO_4.—The concentrated sulfuric acid used was prepared by boiling Baker's C.P. concentrated sulfuric acid a short time in order to remove any dissolved fluorine and SO_2. The SO_2 interferes in the colorimetric determination only by necessitating the use of a larger quantity of hydrogen peroxide than is ordinarily used in the procedure. The acid is stored in a ground glass stoppered bottle to prevent absorption of moisture. It was found unnecessary to have the acid as concentrated as 98.5 per cent, as is advocated by Wagner and Ross (8). This point was tested by using a lower concentration of acid and also by using various amounts of calcium oxide in the sample. It was found that 90 per cent acid was just as effective as the 98.5 per cent in a corresponding temperature range up to 200°; however, above 200°C. water is given off and upon condensing in the delivery tube interferes with the determination.

From the equation: $CaO+H_2SO_4=CaSO_4+H_2O$ it can be calculated that nearly 3 grams of calcium oxide or its equivalent are necessary to react with five cubic centimeters of 98.5 per cent sulfuric acid to reduce it to 90.0 per cent.

Silica.—The silica used was Arthur H. Thomas's commercial silicic acid powder. Before use the silicic acid was boiled with concentrated sulfuric acid to remove any fluorine that might be present.

Standard titanium solution.—The standard used is empirical. A standardized twenty per cent solution of titanous chloride was diluted, 2.5 cc. to one liter, in 3 per cent sulfuric acid. The acid is necessary to prevent hydrolysis of the titanous chloride which takes place readily in a neutral or only slightly acid solution. A stronger acid (5–10 per cent sulfuric) will cause a darker yellow color upon addition of hydrogen peroxide but it is

not bleached to as great an extent by fluorine. This method of using a titanous solution to make up the standard is much simpler than the method of dissolving titanium dioxide as described by Stieger (10). Stieger recommends the use of a titanium solution containing 0.001 gm. titanic oxide per cubic centimeter. The above solution contains the equivalent of 0.000258 gm. titanic oxide per cubic centimeter which, when diluted as described in the procedure, gives a concentration equivalent to 0.0000516 gm. titanium oxide per cubic centimeter as the final dilution.

Hydrogen peroxide.—The hydrogen peroxide used was Baker's Analyzed C.P. 3 per cent. It was found to contain a small amount of fluorine but the amount needed for the oxidation was so small that the fluorine present was treated as a constant factor and thus played no rôle in the determination. The C.P. 3 per cent solution was diluted 1–5 before use in order to reduce the error in the measurement of small quantities of the liquid.

Apparatus

The apparatus required for the volatilization process consists of the following individual pieces assembled in series:

(a) Two gas-washing bottles containing concentrated sulfuric acid to remove moisture from the air which is drawn through the apparatus during the determination.

(b) A cylinder loosely packed with glass wool and phosphorous pentoxide, also for removing moisture.

(c) A reaction tube for digesting the sample with concentrated sulfuric acid. This tube is an ordinary test tube with a side arm delivery tube. Air is introduced through a piece of glass tubing which runs down the center of the tube to the bottom, being led in through a rubber stopper. By using a T tube the acid is also led in through this same tube from a dropping funnel without disturbing any part of the apparatus. The air delivery tube is slightly bell shaped at the bottom so as to give larger bubbles and thus keep the contents in the rounded bottom portion of the reaction tube well agitated. (d) A furnace, with rheostat, for heating the reaction tube. A type 84, Eimer and Amend multiple unit electric furnace was used. (e) An absorption tube which consists of a small test tube fitted with an inlet tube which has been drawn out to a large capillary. The outlet tube is connected with a water aspirator through a pressure regulating vessel containing mercury. (f) The colorimeter used was a Leitz, Bürker type. The advantage of this instrument in this case is that two cubic centimeters of solution are sufficient for a determination.

Ashing

The material to be tested for fluorine by the method used must, if it contains organic matter, be ashed by a suitable method. Fluorine is easily volatilized and thereby lost, so that extreme care must be exercised during this part of the procedure. It was found that there are three major potential sources of error in ashing. They are (a) high temperature, (b) acid ash, and (c) moisture in the sample when it is placed in the furnace.

The treatment of the sample before ashing depends entirely upon the nature of the substance. Bones and teeth were dried and ashed directly. Biological materials which gave an acid ash (usually containing a large amount of phosphorus) were mixed with calcium acetate if only slightly acid and with sodium hydroxide and calcium acetate if they gave a very acid ash, e.g. such as casein. The calcium added serves to make the ash alkaline and also acts as a catalyst in the combustion of the carbon. A number of other metals, cerium, lanthanum and thorium were tried as catalysts, as is advocated by Walters (11) in the ashing of flours, but were found to be inferior to calcium under the conditions necessary in this determination. The sodium hydroxide and calcium acetate were added to the sample in solution to insure a complete and uniform distribution of these two substances throughout the sample. The sample was then dried in an oven at about 80°C. before being ashed. It was found that if drying were not complete before the sample was placed in the muffle furnace, fluorine added as sodium fluoride was lost. If plant materials or any material containing boron in the form of borax or boric acid and probably other boron compounds are to be tested for fluorine by the volatilization of silicon tetrafluoride, it is necessary to add sodium carbonate to the sample before ashing to prevent the formation of a non-volatile boron fluoride compound.

Ashing was carried out in porcelain dishes placed in a muffle furnace at a temperature not exceeding a dull red heat (500°–600°C.). With some ashes a temperature much higher (700°–800°C.) will not volatilize an appreciable percentage of the fluorine but this is not true in all cases. Glazed porcelain dishes were found to be very satisfactory for ashing. The time necessary for complete ashing varies with the sample but if the ash is sufficiently alkaline the ashing will be complete in from two to three hours. Some difficulty in obtaining complete ashing is sometimes experienced with samples having a high phosphorus content. This difficulty, however, is easily overcome by increasing the amount of calcium added before ashing is begun.

The ash after being thoroughly mixed with a quantity of silica powder

is ready to be placed in the reaction tube. The quantity of silica will depend upon the amount of ash,—for one gram of ash 0.2 to 0.3 gm. of silica is sufficient; however, excess of silica does not seem to interfere in any way with the determination. The quantity advisable to use depends more on the total volume in the reaction tube than upon the size of the ash sample.

Volatilization of Fluorine as Silicon Tetrafluoride

The ash intimately mixed with silica as described above is placed in the reaction tube. It was found that if the reaction tube and delivery tube were dried at 80°–100°C. just previous to a determination, the percentage recovery was much greater and more constant (see Table I). Using sodium fluoride as the test material it was found that 70–90 per cent could be recovered (see Table I) if moisture were kept from all parts of the apparatus that came in contact with the silicon tetrafluoride gas.

Table I

Colorimetric Determinations to Compare Values for Fluorine from Pure NaF and Volatilized F

Amount F	Not volatilized	Volatilized in oven dried apparatus	Volatilized in air dry apparatus
Mg.	Reading	Reading	Reading
0	5.00	5.00	5.00
0.01	5.25	5.19	5.00
0.02	5.49	5.40	5.11
0.03	5.75	5.64	5.28
0.04	5.96	5.88	5.34
0.05	6.25	6.13	5.60
0.06	6.52	6.42	5.82
0.07	6.80	6.69	5.90
0.08	7.03	6.92	6.00
0.09	7.28	7.00	6.10
0.10	7.32	7.12	6.20

The reaction tube is then connected in place in the furnace and a stream of air drawn through the apparatus at such a rate that bubbles are formed at the end of the capillary delivery tube. Concentrated sulfuric acid—10 cc. for samples up to one gram and correspondingly more for larger samples—is then added through the air inlet tube, the rate of addition depending upon the nature of the sample. The temperature of the reaction tube is raised to 190°–200°C., kept there for one and one-half hours and then gradually lowered. The temperature is observed by means of a thermometer with the bulb placed about midway of the sulfuric acid column. It is

very necessary that the temperature be kept below 200°C., so that phosphoric acid will not be volatilized and carried over. The air drawn through the apparatus serves the following three purposes, (a) to sweep the silicon tetrafluoride formed over into the absorption tube, (b) to keep the sample and the silica constantly and thoroughly mixed and (c) as a mechanical agitator to prevent the trapping of minute bubbles of silicon tetrafluoride. The flow of air is regulated in such a way as to avoid the necessity of mechanical shaking of the reaction tube. The time necessary for a complete determination is 2–2 1/2 hours.

The absorption tube, as stated above, is small, two cubic centimeters of water being used as the absorbing medium. Sodium hydroxide was tried as the absorbing medium when a large amount of chlorine was present in the sample but was found to be unnecessary, as would be expected when the fact that fluorine replaces chlorine in solution is borne in mind. When the volatilization is complete the absorption tube is disconnected, the contents accurately measured and placed in a test tube. Sufficient water is then added to bring the total volume up to 3.5 cc. and it is ready for the colorimetric determination.

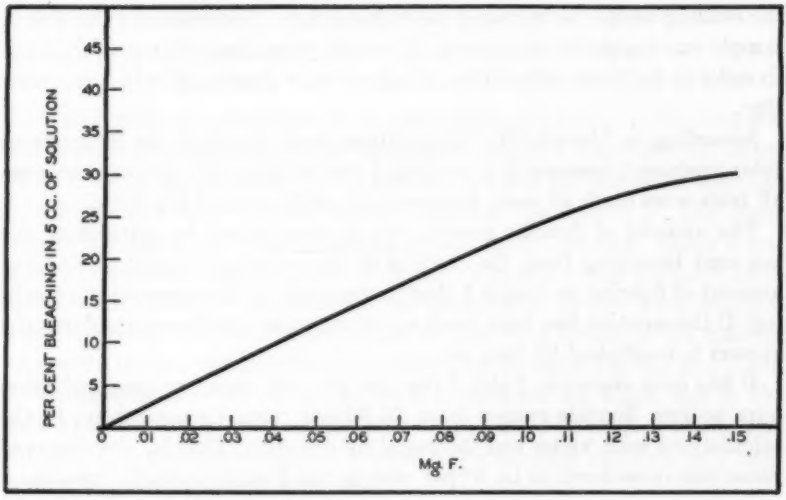
Colorimetric Determination

The colorimetric determination, as stated above, depends upon the bleaching action of fluorine on the yellow color obtained when a solution containing titanium is oxidized by means of hydrogen peroxide. However, the per cent of color change, calculated as per cent bleaching, plotted against amount of fluorine present, does not follow a straight line after a 20 per cent bleach is obtained. Consequently a curve must be constructed as shown in Graph 1.

The Bürker colorimeter is constructed so that the standard solution always has an effective stratum thickness of 10 mm. Thus, a solution of the same strength as the standard would give a reading of 10, the scale being graduated in millimeters. However, since the nature of the construction of the instrument causes the range above the 10 mm. mark to be limited, and since all readings would be less than the standard, it was deemed advisable to use a solution in the "standard" cup of just one-half the strength of the solution used in the determination, thus giving a reading of 5.0 mm. for the standard solution. This relationship was used throughout the experiment. This method also provides a very simple way of determining the per cent of bleaching. It is obvious that a reading of 10 mm. would mean that half the color had disappeared or, in other words, it is a fifty per cent bleach; a reading of 6 mm. is a 10 per cent bleach, etc.

The concentration of the titanium solution chosen is such that it gives a light yellow color, yet concentrated enough to give a definite color in the colorimeter. This concentration was chosen for this reason rather than for any stoichiometrical relationship.

The procedure for the colorimetric determination is as follows: The solution containing the fluorine is made up to a volume of 3.5 cc. as stated



GRAPH 1.

above; 1 cc. of the standard titanium solution and 0.5 cc. of the 1–5 dilution of hydrogen peroxide is added, the total volume now being 5.0 cc. Part of this is used to rinse the cup and plunger of the colorimeter (1.5–2.0 cc. being sufficient for a determination). Ten individual readings are made and the average is taken as the value for that particular determination.

CALCULATION

In order to arrive at a correct figure for this fluorine process a few corrections must be applied. Stieger (10) called attention to the fact that the percentage bleaching is not proportional to the amount of fluorine. It was also found to be true in this work (see Graph 1). However, it will be seen from the graph that the per cent bleaching is proportional to the amount of fluorine present in the range from zero to twenty per cent. Thus, if the volume of solution is controlled so that the per cent bleaching will be less than 20 per cent it can be seen from the graph that the determination will,

in all probability, permit a more delicate determination. That is, the system produces a greater change in color per unit amount of fluorine up to a 20 per cent bleach. With this in mind, dilutions of the test solution were always made with the "5 mm. reading" standard when there was more than 20 per cent bleaching. However, it can readily be seen that there would be little advantage in diluting more than three times since—the calculation being made on a 5.0 cc. volume basis—the error introduced in the reading would be increased correspondingly. Consequently the size of sample was regulated so as never to contain more than 0.2 mg. of fluorine. In order to facilitate calculation, dilutions were always made in 5 cc. quantity.

According to Merwin (9), temperature plays a part in the intensity of color produced; however it is small and can be ignored in this case because all tests were made at room temperature, which varied but little.

The amount of fluorine present can be determined by calculating the per cent bleaching from the reading on the colorimeter and finding the amount of fluorine on Graph 1 that corresponds to that amount of bleaching. If the solution has been made up to ten cubic centimeters volume the answer is multiplied by two, etc.

It has been shown in Table I that the per cent recovery expected using pure sodium fluoride ranges from 70–90 per cent. Consequently, in the calculations each value was increased by one-fifth. That is, the observed value was considered to be 80 per cent of the fluorine actually present.

Accuracy of the Method

That the method is accurate within reasonable limits of error for such small quantities of material involved is shown by the fact that a nice range of values can be obtained with differences of but 0.01 of a milligram and that a constant and large per cent of the fluorine can be recovered when NaF is added to a sample. Check results are also obtained when a sample having an unknown amount of fluorine is used. Table II shows some duplicate results on bone ash samples.

Table II
Fluorine Determinations to Show Ability to Duplicate Values

Sample	Wt. Sample	Dilution	Reading mm.	Per cent F.
11	0.4172	5.0	5.53	0.0059
11	0.6779	5.0	5.80	0.0054
8	0.2218	10.0	6.89	0.0834
8	0.1438	5.0	7.04	0.0766

Preparation of Low Fluorine Diet

Salts.—The individual salts of McCollum's salt mixture No. 51, which includes the following salts in proportions shown, were tested for fluorine.

$CaCO_3$	1.5
KCl	1.0
$NaHCO_3$	.7
NaCl	.5
MgO	.2
Fe citrate	.5
KH_2PO_4	1.7

Each salt, with the exceptions of the phosphate and ferric citrate, was dissolved and tested directly by the colorimetric method. They were all tested by an etching method also. The potassium and sodium chlorides were also tested by the sensitive color reaction described by Boer (12). The ferric citrate was ashed and tested by the volatilization method. Baker's Analyzed Chemicals were used and, with the exception of the primary phosphate, were found by the above named methods to be free from fluorine. The phosphate which interferes with the bleaching action of the fluorine was tested by the volatilization procedure. Considerable fluorine was found in one sample; however, a sample from a different lot which was in the form of fine crystals, whereas the first lot consisted of large crystals, was found to give a negative test even when a special large reaction tube containing 10 gm. of the salt was used.

Dextrin.—The dextrin used in the stock ration in this laboratory was found to contain considerable fluorine. The starch from which the dextrin was made was also found to contain fluorine; however, the absolute amount was quite variable and some samples contained none. This suggested that the fluorine was held in a very loose way and that it might be possible to wash it out of the starch with acidified water. Such a method was tried and was found to remove the fluorine completely insofar as could be determined in the ash from 100 grams of material. The final procedure adopted was as follows: one-half pound of corn starch[1] per liter of three per cent HCl, by volume, was thoroughly churned by an electrically-driven stirrer for from two to three minutes, then the starch was allowed to settle, leaving a clear solution above. The clear supernatant solution was then drawn off and the process repeated three times with distilled water. The starch was dried in pyrex dishes in a warm air oven and then pulverized.

[1] Argo brand.

Other constituents.—The yeast used in this laboratory[2] was found to be free from fluorine insofar as could be determined. That is, less than 0.02 mg. per 100 gm. or less than one part per five million was present.

Butterfat, made by melting butter and filtering off the curd and water, also gave a negative test for fluorine in the ash from 100 grams.

Viosterol[3] was also tested and gave a negative test in the ash from ten cubic centimeters.

TABLE III

COMPOSITION OF DIETS

Constituents	Test Diet	Control
Casein	18.0	18.0
Butterfat	8.0	8.0
Salts 51A*	6.5	6.5
Yeast	10.0	10.0
Starch	57.5	57.5
Viosterol	15 drops/kilo.	15 drops/kilo.
Sodium fluoride		0.003

* Composition of salts 51A:

$CaCO_3$	150.0 grams
KCl	100.0
NaCl	50.0
$NaHCO_3$	70.0
Ferric citrate	50.0
KH_2PO_4	170.0
$MgSO_4$	60.0

Casein as prepared in the laboratory by washing the commercial product with acetic acid and then with distilled water was found to contain a small amount of fluorine. However, a sample of the commercial product before washing gave a negative test for eight individual determinations using 50 grams for each. The material was also tested by adding 0.1 mg. of fluorine to some samples as sodium fluoride, then ashing and testing for fluorine. The calculated amount of fluorine was recovered in each case. From this it was reasoned that there was nothing present interfering with the determination and that being the case there was no fluorine present.

Diet formulae.—With the above salts and organic materials the experimental diets adequate in all the factors known to be required for growth and reproduction were made up. The composition of the diets is shown in Table III.

[2] Northwestern Yeast Company's dried yeast.

[3] Mead's Viosterol 250D.

Care of the Rats

The rats used in this investigation were the McCollum strain which has been inbred for over eighteen years. The rats were kept in a special room where no roach powder was used. Sixteen rats from three different litters, about thirty-five days of age, and weighing about 50 gm. each, were chosen for the experiment. Ten rats, five males and five females, were fed the low fluorine ration, while six, three males and three females, were fed the same ration with 0.001 per cent fluorine added. The rats had access to the ration at all times. Distilled water also was kept before them at all times. Once a week iodine (4.2 mg. per 100 cc.) was added to the drinking water to insure an ample supply of that element. The rats were all weighed once a week. Females were placed in individual cages when they became pregnant and were given filter paper clippings for nest material (this was found to be free from fluorine).

Table IV

Reproduction Record (Total Litters)

Series	Second Generation			Third Generation		
	Number of litters	Number of rats	Number rats lived	Number of litters	Number of rats	Number rats lived
Low F	8	64	24	9	63	5
Control	5	46	7	5	30	0

Calcium and Phosphorus Determinations

Calcium determinations were made by McCrudden's (13) method, which consists of the precipitation of calcium oxalate and titration with potassium permanganate. Phosphorus was determined by the volumetric method of the A. O. A. C. (14). Determinations were made upon a solution of the ash of the bones. From 0.3 to 0.5 gm. of ash were dissolved in dilute nitric acid and the solution made up to a volume of 200 cc. Aliquots were taken of this stock solution for both the calcium and phosphorus determinations.

Calcium and phosphorus determinations were made on the bones only. All the teeth were used for fluorine determinations or for histological study. Determinations were made on the combined ashes of tibiae, fibulae, femurs and humeri. Calcium determinations were made in triplicate and phosphorus determinations in duplicate.

TABLE V
REPRODUCTION RECORD (FIRST LITTERS)

Series	Second Generation			Third Generation		
	Number of litters	Number of rats	Number rats lived	Number of litters	Number of rats	Number rats lived
Low F	5	43	12	8	58	0
Control	3	27	0	5	30	0

TABLE VI
FLUORINE AND ASH CONTENT OF TEETH AND BONES

Description	Teeth		Bones	
	% Ash	% F in Ash	% Ash	% F in Ash
Stock animals				
2 yr. old females			60.9	0.051
1 yr. old females	68.20	0.018	60.1	0.061
12–18 mo. old	69.9	0.021	61.4	0.075
Young males	66.5	0.0025	58.5	0.070
35 days old	65.7	0.006	49.2	0.02
45 days old	64.80	—	47.5	0.035
28 days old	61.6	0.000?	45.9	0.00?
Not stock, but normal 50 days old (on an experimental diet not analyzed)			50.69	0.01
Low F Animals				
(4 mos.)	Lower 73.7	<0.0005	64.9	0.015
	Upper 71.5	<0.0005		
Diet No. 2				
Male, 3½ mos.			60.02	0.0046
Female, 4½ mos.			60.09	0.0046
Male, 4½ mos.	75.33*	<0.0005	61.86	0.0027
2nd. gen. 8 wks.			58.74	0.001
2nd gen. 3 mos.			63.3	0.0045
Diet containing .001% F added				
Female, 3½ mos.			65.72	0.120
Male, 3½ mos.			65.11	0.056

* Combined to give a larger sample.

Observations on the Experimental Animals

Gross Observations.—The rats on the low fluorine diet looked very well, were quite fat and on the whole appeared normal. No difference could be noted between these and the control rats which were fed fluorine. The hair of the low fluorine rats was perhaps a little coarser than that of the control rats but not enough to be striking. The incisors of both series of animals were of a deep orange color on the ventral surface. The superior incisors were darker than the inferior. The teeth showed no caries and from gross appearances seemed to be perfect.

Table VII
Calcium and Phosphorus Content of Rat Bones

Substance	% Ash	% F	% Ca	% P	Ca/P
Bones, female, F fed	65.72	0.12	37.21	17.81	2.09
Bones, male, F fed	65.11	0.056	36.97	17.70	2.09
Bones, male, low F	61.86	0.0027	38.61	18.23	2.12
Bones, female, low F	60.09	0.0046	37.79	18.24	2.07
Bones, males, low F	60.37	0.003	36.88	18.42	2.00
Bones, males and females, low F	58.74	0.001	36.67	18.42	1.99
Bones, normal young	50.69	0.01	35.64	19.45	1.83
Bones, 2 low F	60.02	0.0046	37.43	18.11	2.07
Bones, normal old females	60.12	0.061	36.92	17.20	2.15
Bones, 1 yr. old females	60.85	0.051	36.97	17.20	2.15
Bones, low F, females	63.30	0.0045	37.63	18.07	2.08
Bones, normal young	47.48	0.035	35.40	18.74	1.89

Reproduction was quite normal. The litters varied in size from five to ten young; the young seemed normal when born and weighed from four to five grams each. The mother in almost every case accepted the young and made a nest in the filter paper clippings provided. However, in many instances the first litter and in some instances the second litter died in from one to two days. Presumably it was starvation since no milk could be seen in the young. However, in some cases the young would obtain milk, live for three or four days and then die. The control animals behaved in the same way. Table IV shows the number of litters, the numbers of rats born and the number that lived in the second and third generations. Table V shows the number of first litters, the number of rats born and the number of rats that lived in the second and third generations.

Many of the rats on the low fluorine diet lost their tails when quite young. The tail would become constricted and the end would either dry up and drop off or would become oedematous and finally break off at the

constriction. This phenomenon did not appear in more than about half the rats on the low fluorine diet and was not observed at all in the controls. The phenomenon was seen only in rats one to three weeks of age. It has been occasionally observed in the stock colony of the laboratory and can hardly be related to fluorine deficiency.

Fluorine Determinations.—Fluorine determinations were made on the teeth and bones of stock rats, of rats on low fluorine diet and of rats on the low fluorine diet with 0.001 per cent fluorine added. Fluorine determinations were also made on stock rats of different ages in order to find out whether or not fluorine accumulates in the bones and teeth. Determinations were made on experimental rats which corresponded as nearly as possible in age with the stock rats used. Determinations were also made on both the first and second generations of the experimental rats. The results are tabulated in the following table (Table VI) which also shows the per cent of ash. All fluorine determinations are reported as per cent of the ash.

Calcium and Phosphorus Determinations.—Calcium and phosphorus determinations were made on the ash of the bones of stock rats of different ages and of the experimental rats. All calcium determinations were made in triplicate and phosphorus determinations in duplicate. The results are shown in Table VII, together with the per cent fluorine and the calcium: phosphorus ratio. Calcium and phosphorus determinations were not made on teeth because of lack of material.

The gross appearance of the teeth of the fluorine-free animals showed no deviation from the normal.

Discussion of Results

The diet was very low in fluorine but not quite free, as shown by the analyses of the bones and teeth.

Much trouble was experienced in getting the young to live. Moreover, fluorine could not be the cause of death since the control rats receiving fluorine behaved in the same manner as did the rats on the fluorine-free ration. In fact the results shown in Tables IV and V even favor the absence of fluorine. It would seem that there was some factor lacking or some factor present that produced a condition which was not favorable for the survival of the young.

Table VI shows that there is normally present a relatively large amount of fluorine in the bones and teeth of the rat, there being more in the bones than in the teeth.

The diet plays a large part in determining the fluorine content of the

bones. The bones of rats on an experimental diet, not analyzed but adequate in every respect, gave a value of only 0.01 per cent fluorine while those on the stock diet gave values of nearly 0.04 per cent for a corresponding age. Bones of rats on the experimental low fluorine diet gave values of 0.001 to 0.0046 per cent or approximately 6 to 25 parts per million of the dry bone. The fluorine content of the bones of rats on the stock diet seemingly does not rise above 0.08 per cent. It will also be seen that as small a quantity as one milligram of fluorine per hundred grams of food added to the low fluorine diet causes as much or more fluorine to be deposited in the bones as does the stock diet.

The values found for the fluorine content of the teeth are extremely interesting in view of the fact that it is in the teeth that the first and most important effects are noticed when an excess of fluorine is fed in the diet. It will be seen from Table VI that there is normally in the stock rats somewhat less fluorine in the teeth than in the bones. Furthermore, no fluorine could be detected in the teeth of the animals on the low fluorine diet. Thus, if fluorine is present as a factor in the consolidation of the tooth, abnormalities should appear in the histological picture. There is, however, no marked abnormality in the structure. The teeth seem to be excellent, no indication of caries, and seemingly perfect calcification. There is some slight indication of hemorrhage and a proliferation of capillaries in the bone just outside of the outer enamel epithelium. If such is the case, there should be some indication of abnormalities in the long bones and in the marrow. Work is being continued along that line.

The calcium and phosphorus determinations shown in Table VII do not show anything striking. The calcium to phosphorus ratio which has been shown to change slightly with the feeding of an excessive amount of fluorine cannot be said to have changed at all. The ratio is slightly higher than that reported by McClure and Mitchell, but it is practically the same for the rats on the stock diet, on the low fluorine diet and on the diet containing added fluorine.

Conclusions

1. A modified method for the colorimetric determination of fluorine is presented.
2. Young rats (16–18 days) contain little if any fluorine.
3. Rats normally contain considerable fluorine in their bones and teeth.
4. Within certain limits the fluorine content of bones and teeth increases with age.
5. The fluorine content of teeth and bones varies with the diet fed.

6. Rats grow normally on a diet low in fluorine.

7. A diet low in fluorine does not affect reproduction in any way.

8. The fluorine content of bones can be reduced to between six and twenty-five parts per million and can be eliminated from the teeth, insofar as can be determined, without showing any gross deleterious effect.

9. There is no striking structural change in teeth free from fluorine from rats on a low fluorine diet.

10. There is some slight indication of a proliferation of capillaries in the tooth pulp and surrounding bone. A similar condition has been at times noted even in the stock animals.

11. There is no change produced in the calcium to phosphorus ratio in the bones by feeding a diet very low in fluorine.

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STUDIES ON GROWTH

I. GROWTH FACTORS IN LIVER*

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THE known facts regarding the nutritive requirements of man and of the lower animals have increased remarkably within the past decade. However, this newer knowledge has not yet made possible a complete understanding of normal nutrition so that at the present time one is still unable to state definitely the nature of an adequate food mixture. The experimental data of investigations in which the white rat has been used have been made particularly confusing by the changes which have been observed recently in the rate of growth of stock or control animals (1, 2, 3). Obviously, results become increasingly difficult to interpret as the standard growth curves or norms vary. An additional complicating factor in some cases has been the acceptance of a fair degree of growth of rats on an experimental diet as satisfactory even though the rate of growth was considerably below that of stock or control rats. The assumption has been made in such cases that artificial mixtures of purified food materials must of necessity be inferior to a mixture of natural foods. It appeared desirable to test the validity of this assumption and, therefore, an attempt has been made to find a satisfactory combination of supplements which would permit optimum growth on a simple basal diet. Inasmuch as liver has been shown by Osborne and Mendel (4, 5) to exert a pronounced stimulating effect upon growth, the experiments have centered around the use of this material as a dietary supplement and an investigation is being made of its water-soluble growth factors.

Twenty different combinations of supplements were tested and all but one permitted a rate of early growth as good or better than that previously recognized as normal (6). It may be stated that the capacity of a supple-

* Preliminary reports of this work were presented before the American Society of Biological Chemists at Montreal, April, 1931 (Graham, C. E. and Griffith, W. H., *Jour. Biol. Chem.*, 1931, **92**, *63*, and before the Society for Experimental Biology and Medicine, St. Louis (Graham, C. E. and Griffith, W. H., *Proc. Soc. Exp. Biol.* and *Med.*, 1929, **26**, 715; 1929, **26**, 862; 1931, **28**, 756; 1931, **28**, 1086).

ment to stimulate appetite was found to be the principal cause of differences between the various groups. This is in agreement with the conclusion of Palmer and Kennedy who, although unsuccessful in devising a synthetic diet equivalent to a natural diet (7, 8), showed that the difference between the two diets depended upon consumption of food and not upon utilization of food (9). The experiments reported in this paper not only demonstrated the possibility of obtaining optimum growth on a simplified diet but they also emphasized the importance of certain dietary essentials in accelerating the rate of growth through a stimulation of appetite.

Experimental Procedures

The simplified food mixture introduced by Evans and Burr (purified casein 25; commercial sucrose 75; salt mixture 4) (10) was chosen as the basal diet. The Osborne and Mendel salt mixture (11) was used and was modified by the addition of 0.2 gm. of $CuSO_4 \cdot 5H_2O$ per kilo. of salt mixture. Norwegian cod liver oil was employed as a source of vitamins A and D. In a few experiments wheat germ oil, prepared by the ether extraction of wheat embryo, was used. The water-soluble vitamins were furnished by one or more of the following: dried starch-free baker's yeast; dried hog liver; dried autoclaved hog liver; tikitiki, an extract of "rice polish" prepared according to Wells (12); yeast extract, an extract containing the antineuritic factor prepared according to Seidell (13); and, an aqueous extract of hog liver.[1] The autoclaved hog liver was prepared by heating fresh hashed liver at 120°C. and 15 lb. for five hours. At the end of this operation the pH ranged from 4 to 5. This material, as well as the whole liver and whole yeast, were dried in a warm air dryer at 50°C.

Young male rats were used, averaging 28 to 30 days of age and 49 to 51 gm. in weight. The rats were kept in individual raised cages except when stated otherwise. Twenty different combinations of supplements were investigated (Table I), each variation being tested on a group of ten or more rats. With one exception no changes were made in the supplements furnished a group during the course of an experiment. The experimental periods ranged from 60 to 200 days. The dry basal diet was fed *ad libitum* in small dishes resembling the McCollum feeding cup and wasting of food was not encountered. The supplements were fed daily in Syracuse dishes.

The consumption of water was measured as well as the gain in weight and the consumption of food. Water was supplied from inverted bottles having delivery tubes with an inside diameter of 5 mm.

[1] We wish to thank Mr. P. A. Katzman for the preparation of the liver extract.

TABLE I

COMPOSITION OF SUPPLEMENTS SUPPLIED THE GROUPS OF MALE RATS

Group	No. of rats	Average starting		Cod liver oil	Yeast	Additional daily supplements
		Age (days)	Weight (gm.)	(gm.)	(gm.)	
1	10	29	51	0.09	0.50	—
2	14	29	53	0.27	0.50	—
3	10	30	51	0.36	0.50	—
4	10	30	50	0.09	0.50	0.18 gm. of lard
5	9	27	51	0.27	0.50	0.09 gm. of lard
6	10	29	53	0.27	0.50	0.09 gm. of wheat germ oil
7	10	30	50	0.27	0.50	10% of lard in basal diet
8	14	31	52	0.27	0.50	(Cages on shavings)
9	10	31	51	0.27	0.50	(Commercial casein in basal diet)
10	10	31	55	0.27	0.50	0.25 cc. of tikitiki
11	19	27	52	0.27	1.00	—
12	10	31	51	0.27	1.00	0.5 cc. of liver extract
13	10	30	50	0.27	0.50	0.5 cc. of liver extract+0.09 gm. of wheat germ oil.
14	10	29	55	0.27	0.50	0.5 gm. of whole dried liver
15	10	30	49	0.27	0.15	0.85 gm. of whole dried liver
16	10	28	49	0.27	0.00	1.0 gm. of whole dried liver
17	12	27	52	0.27	0.00	0.21 gm. of autoclaved liver+0.25 cc. of tikitiki
18	12	28	52	0.27	0.00	1.0 gm. of autoclaved liver+0.25 cc. of tikitiki
19	10	29	52	0.27	0.00	1.0 gm. of autoclaved liver+0.5 cc. of yeast extract.
20	10	29	51	0.27	0.50	1.0 gm. of autoclaved liver
21	10	29	54	0.00	0.00	(Stock diet)

The caloric value of the total ingested food was calculated on the basis that the basal diet, yeast and liver furnished 3.75, 2.5 and 3.75 calories per gram respectively. Supplements of cod liver oil, lard and wheat germ oil were included on the basis of 9 calories per gram. Supplements of tikitiki, liver extract and yeast extract were not included in the calculation of the caloric intake.

The coefficients of digestibility of the stock diet and of the adequately supplemented basal diet were determined according to the method of Armsby (14) in which the ratio of dry feces to dry food is found. The results showed the stock and basal diets to be 76.5 and 98 per cent digestible respectively. On the basis of these results the necessary correction was made for the caloric intake of the rats on the stock diet and the experi-

mental diet was assumed to be wholly digestible. The stock diet used in our colony is a commercial dog biscuit which has been found adequate for growth, reproduction and lactation.

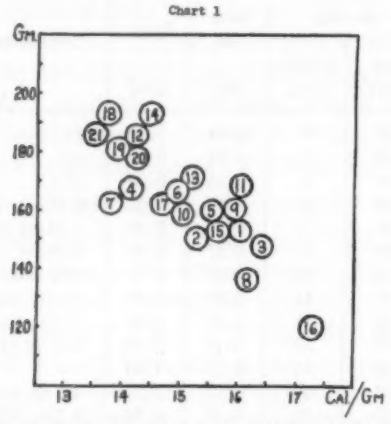

CHART 1. The average gain in weight during the first 60 days (30 to 90 days of age) of groups listed in Table I plotted against calories required per gram gain in weight.

The extensive data obtained in these experiments are summarized in Tables I to V and Charts 1 to 3. The results are presented as group averages since it was obviously impracticable to give the complete data for each

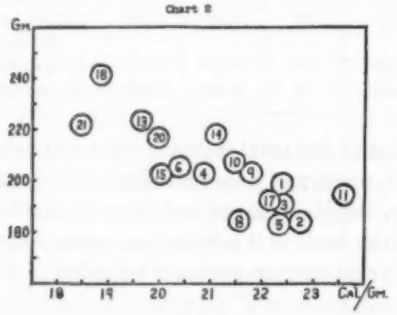

CHART 2. The average gain in weight during the first 100 days (30 to 130 days of age) of groups listed in Table I plotted against calories required per gram gain in weight.

of the 270 rats. It was recognized that larger groups would have been more desirable in view of the individual variations which appear even among litter mates. However, it was believed that the conclusions which were

drawn from the data were significant since they were not dependent upon the results of any one group.

Although a record of the gain in weight and of the food and water intake was made for each rat for every 10 or 20 day interval, the results were combined in the tables into three periods, the first 60 days, the first 100 days and the period exceeding 100 days. The 60 day period represented the period of most rapid growth while the 100 day period represented the period of rapid growth and the attainment of nearly adult size. For these

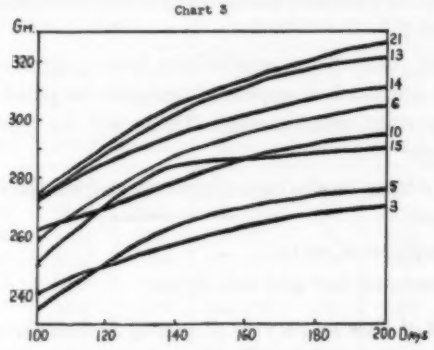

CHART 3. The average growth curves during the second 100 day period (130 to 230 days of age) of some of the groups listed in Table I. The number of the group is indicated on each curve.

two periods the efficiency of utilization of food was expressed in terms of calories required to increase one gram in weight. The period exceeding 100 days represented an interval of slow growth in which a much higher proportion of the ingested food must have been used for maintenance than was the case in the periods of rapid growth. For the later period the ratio of calories to gain in weight has little significance. In order to compare the utilization of food under the influence of the various supplements during this third period, two "efficiency quotients" were calculated. E.Q.I represented the calories consumed per day per 100 gm. of body weight and was calculated as follows:

$$\text{E.Q.I} = \frac{\text{Total calories} \times 100}{\text{Mean weight} \times \text{Length of period in days.}}$$

E.Q.II represented the calories consumed per day per gram of gain in weight per 100 gm. of body weight and was calculated as follows:

$$\text{E.Q.II} = \frac{\text{Total calories} \times 100}{\text{Gain in weight} \times \text{Mean weight} \times \text{Length of period in days.}}$$

E.Q.II was patterned after that suggested by Palmer and Kennedy (9) and differs only in the use of total calories per day in place of total grams of dry food.

It was assumed in the consideration of the results that vitamins B and G were the water-soluble vitamins required by the rat since only these two have been definitely established as members of the B complex (15, 16). The autoclaved liver was found to be a rich source of vitamin G and was probably free from vitamin B.[2] The tikitiki, as was shown by Evans and Burr (17, 18), was an excellent source of B which contained not more than a trace, if any, of G.[2]

Although many interesting possibilities were suggested by the results which have been obtained, it appeared desirable to point out only certain of the more important relationships. These will be discussed under the following headings:

1. The effect of liver on the rate of growth and on the consumption and utilization of food.
2. The consumption of water.
3. The occurrence of feet and tail signs.

1. The effect of liver on the rate of growth and on the consumption and utilization of food

Whole dried liver (1.0 gm. daily) was not an adequate source of the water-soluble vitamins although this supplement permitted some growth (Table II, Group 16). Substitution of 0.15 gm. of the liver with an equal quantity of yeast (Group 15) improved growth but not so much as substitution of half of the liver with yeast (Group 14). It was evident that the limiting factor in liver was B since excellent growth resulted if the supplement contained autoclaved liver and a source of B such as tikitiki (Group 17 and 18) or yeast extract (Group 19). Group 18 grew as well as the control group (Group 21) which indicated that 1.0 gm. of autoclaved liver was adequate as a source of G during the first 100 days on the experimental diet.

The effect of liver in accelerating the rate of growth is clearly shown in the last column of Table II which gives the number of days required to attain a weight of 200 gm. from a starting weight of approximately 50 gm. The only groups which required less than 50 days were those receiving liver in some form (Groups 12, 13, 14, 18, 19 and 20). The only groups which received liver and required more than 50 days were the two groups

[2] Unpublished experiments.

mentioned above as being definitely low in the supplement of B (Groups 15 and 16) and Group 17, which received only 0.21 gm. of autoclaved liver daily. The significance of this effect of liver becomes more important upon examination of the results with Groups 1 to 11, none of which received liver and all of which required more than 50 days to reach 200 gm.

TABLE II

AVERAGE WEIGHT OF GROUPS OF MALE RATS AND AVERAGE FOOD AND WATER CONSUMPTION DURING 60 AND 100 DAY PERIODS

Group	Results for first 60 days				Results for first 100 days				Days to reach 200 gm.
	Final Weight		Total food	Total water	Final Weight		Total food	Total water	
	Range (gm.)	Average (gm.)	(cal.)	(cc.)	Range (gm.)	Average (gm.)	(cal.)	(cc.)	
1	174–233	203	2405	1093	225–291	249	4438	2116	60
2	188–240	204	2355	1081	206–272	236	4155	2158	62
3	137–245	198	2403	1231	204–304	241	4238	2346	62
4	178–260	217	2375	944	219–305	253	4245	1722	52
5	189–216	203	2397	910	219–265	235	4132	1786	61
6	196–238	217	2452	895	220–290	258	4197	1775	51
7	172–245	212	2235	1033	—	—	—	—	—
8	162–208	187	2195	1268	200–258	236	3980	2424	70
9	175–256	210	2525	1227	208–310	254	4443	2389	57
10	158–258	214	2385	1232	224–326	262	4455	2484	55
11	178–259	218	2670	1292	205–278	247	4600	2368	55
12	209–270	235	2620	1459	—	—	—	—	45
13	194–255	219	2557	1113	249–315	273	4382	2080	48
14	197–287	247	2790	1159	218–316	274	4635	2084	41
15	157–225	204	2415	1045	216–285	252	4105	2009	61
16	148–188	168	2064	914	—	—	—	—	—
17	183–256	215	2403	1136	210–273	245	4309	1930	68
18	199–289	243	2607	1279	249–347	294	4547	2525	44
19	184–272	233	2542	1216	—	—	—	—	46
20	192–282	230	2527	1265	220–331	268	4342	2373	46
21	198–278	241	2540*	1423	224–300	275	4052*	2513	38

* 76.5 per cent of actual caloric intake.

The superiority of the liver supplement is also shown in Charts 1 and 2 in which the 60 and 100 day weight increments are plotted against the calories required to gain one gram in weight. Groups 1 to 11 received a minimum of 0.5 gm. of whole dried yeast as a source of vitamins B and G. This was inadequate for optimum growth. Furthermore, an increase or decrease in cod liver oil (Groups 1, 2 and 3), the addition of lard (Groups 4, 5 and 7), the addition of wheat germ oil (Group 6), access to feces

(Group 8), the use of commercial casein (Group 9), the addition of tikitiki (Group 10) and the doubling of the amount of yeast (Group 11) were all ineffective in bringing about optimum growth.

The optimum early growth due to the presence of liver in the daily supplement was associated with an increased appetite and with an apparently more efficient utilization of food. The data for food consumption for the first 60 days show that nine of the eleven groups which did not receive liver (Groups 1 to 11) consumed less than 2500 calories whereas six of the

TABLE III

AVERAGE WEIGHT OF GROUPS OF MALE RATS AND AVERAGE FOOD AND WATER CONSUMPTION DURING EXPERIMENTAL PERIOD AFTER FIRST 100 DAYS

Group	Experimental period (days)	Mean weight (gm.)	Final weight (gm.)	Gain per day (gm.)	Calories per day	Water per day (cc.)	E.Q.* I	E.Q.** II
3	100–200	256	270	0.29	45.8	25.5	17.9	0.62
4	100–180	260	267	0.18	41.6	19.8	16.0	0.91
5	100–200	256	276	0.41	44.8	21.5	17.5	0.43
6	100–200	282	305	0.47	44.2	21.8	15.7	0.33
10	100–200	279	295	0.33	47.9	27.0	17.1	0.52
11	100–160	268	278	0.33	47.8	30.7	17.8	0.54
13	100–200	297	321	0.48	48.6	23.3	16.4	0.34
14	100–200	292	310	0.36	45.4	24.1	15.5	0.43
15	100–200	271	290	0.38	44.4	24.2	16.3	0.43
17	100–160	252	259	0.23	38.7	24.5	15.3	1.10
18	100–180	302	310	0.19	48.8	22.4	16.1	0.85
20	100–180	276	284	0.20	45.1	26.7	15.9	0.80
21	100–200	301	326	0.51	42.2	29.9	14.0	0.27

$$* \text{E.Q. I} = \frac{\text{Calories per day} \times 100}{\text{Mean weight}}$$

$$** \text{E.Q. II} = \frac{\text{Calories per day} \times 100}{\text{Gain} \times \text{Mean weight}}$$

nine groups receiving liver consumed more than 2500 calories. The three groups receiving liver and consuming less than 2500 calories were, again, the groups furnished supplements which were definitely inadequate (Groups 15, 16 and 17). Inspection of Chart I shows that eight of the eleven groups not receiving liver (Groups 1 to 11) required 15 or more calories per gram of gain in weight while six of nine groups receiving liver required less than 15 calories per gram of gain. It is realized that these figures are not necessarily indicative of variations in the efficiency of utilization of food, because of the number of variables concerned. However,

the data do relate rapid early growth and increased food consumption to the presence of liver in the daily supplement.

The effect of liver was not only evident in accelerating the rate of early growth but was also related to the continuation of growth after the first 100 days. Of the four groups which averaged more than 300 gm. at the termination of the experimental period, three consumed diets containing liver in some form (Table III and Chart 3). Table III shows the average daily food consumption and two efficiency quotients for experimental periods in excess of 100 days. Such quotients may have real value if comparisons are made on rats of the same weight or on rats receiving the same number of calories. The actual significance of the quotients given in Table III cannot be determined until more definite information is available concerning the relative caloric requirements of maintenance and growth. It was of interest to find that of the seven groups which were continued on the experimental diets for 200 days, the average daily caloric intakes were 41.5, 43.1 and 45.9 calories per day for the 60 day, the 100 day and the 100–200 day periods respectively.

TABLE IV

RATIO OF TOTAL WATER CONSUMPTION TO TOTAL FOOD CONSUMPTION $\left(\frac{\text{cc.}}{\text{calories}}\right)$

Group	0 to 60 days	0 to 100 days	Period over 100 days
1	0.45	0.48	—
2	0.46	0.52	—
3	0.51	0.55	0.56
4	0.40	0.41	0.48
5	0.38	0.43	0.48
6	0.37	0.42	0.49
7	0.46	—	—
8	0.58	0.61	—
9	0.49	0.54	—
10	0.52	0.56	0.56
11	0.48	0.52	0.64
12	0.56	—	—
13	0.44	0.48	0.48
14	0.42	0.45	0.53
15	0.43	0.49	0.54
16	0.44	—	—
17	0.47	0.45	0.63
18	0.49	0.56	0.46
19	0.50	—	—
20	0.50	0.55	0.59
21	0.56	0.62	0.71

A specific demonstration of the effect of liver on appetite is seen in the comparison of Groups 17 and 18 which received the same amount of tiki-tiki but different quantities of autoclaved liver. Group 18 consumed more food in all three periods.

2. The consumption of water

The total average consumption of water during the first two periods is given in Table II and the average daily consumption of water in the third period is given in Table III. Table IV shows the water intake per calorie of food consumed for the three periods. It is quite apparent from this table that a close relationship existed between the amounts of food and water consumed. The value of the ratio increased slightly with the age of the rats, the average values for the 7 groups on the experimental diets for 200 days being 0.44, 0.48 and 0.51 cc. per calorie for the three periods.

TABLE V

NUMBER OF RATS SHOWING FEET OR TAIL SIGNS

Group	20 days			60 days			100 days			200 days		
	+	±	−	+	±	−	+	±	−	+	±	−
1	3	4	3	5	5	0	9	1	0	0	1	9*
2	8	1	5	11	3	0	12	1	1	—	—	—
3	5	4	1	9	0	1	10	0	0	1	0	8
4	0	2	8	1	3	6	0	0	10	0	0	10
5	3	1	5	3	4	2	4	2	3	0	2	7
6	2	5	3	0	4	6	0	1	9	0	0	10
7	0	0	10	0	2	8	—	—	—	—	—	—
8	0	0	10	2	7	5	5	7	2	—	—	—
9	0	1	9	7	2	1	9	0	1	—	—	—
10	4	2	4	10	0	0	10	0	0	1	3	6
11	4	4	2	7	3	0	9	1	0	3	4	3**
12	3	5	2	10	0	0	—	—	—	—	—	—
13	1	0	9	0	3	7	0	2	8	0	0	10
14	0	4	6	0	4	6	0	3	7	0	1	9
15	0	2	8	0	4	6	0	4	6	0	0	10
16	0	0	10	0	2	8	0	1	9	—	—	—
17	0	1	11	2	9	1	0	4	8	0	0	12**
18	2	5	4	0	8	4	0	3	9	0	0	12†
19	2	3	5	2	5	3	—	—	—	—	—	—
20	0	6	4	0	8	2	0	4	6	0	0	10†
21	0	0	10	0	0	10	0	0	10	0	0	10

* Rats discontinued at 140 days.
** Rats discontinued at 160 days.
† Rats discontinued at 180 days.

3. *The occurrence of feet and tail signs*

Dermatitis of the feet and tail was of common occurrence among the experimental rats (Table V). The "feet and tail signs" consisted of dry scaliness of the feet, of the hind feet particularly, and scaliness and soreness of the tail. The signs were apparently identical with those described by Burr and Burr (19). In some rats the feet signs appeared first, in others the tail signs preceded the feet signs. In most instances the signs appeared within 20 days, were most severe between 40 and 60 days and slowly disappeared so that they were scarcely evident at 200 days except in those rats which had lost sections of the tail.

The signs were very severe in the rats receiving cod liver oil and 0.5 gm. of yeast daily (Groups 2 and 3) and were not prevented by doubling the yeast (Group 11), by using commercial casein (Group 9), by adding tikitiki (Group 10) or by adding liver extract (Group 12). The signs were absent on the stock diet (Group 21). Wheat germ oil, whole liver, and lard were effective in decreasing the severity of the signs (Groups 6, 15 and 7). The failure of the liver extract to prevent the signs (Group 12) indicated that liver fat was possibly the effective agent in whole liver.

Changes in the feet and tail of the rat have been reported by other investigators and appear to be associated with various types of nutritive deficiencies (20 to 25). Although Burr and Burr originally reported that cod liver oil prevented the signs, they later came to the conclusion that the highly unsaturated fatty acids of cod liver oil were used in low fat diets for growth but that the scaly skin was cured only by linolic or linolenic acids which are apparently lacking in cod liver oil (26). Sinclair also noted that the signs were not prevented by cod liver oil but were prevented by lard and also by permitting access to feces (24).

This particular abnormality did not appear to be related necessarily to vitamin B or G deficiency and might therefore complicate the interpretation of results on low fat, low G diets. In this connection the possible presence of toxic substances in experimental diets may be important. Such injurious compounds have been suggested by the work of Norris and Church (27), Parsons (22) and Fixsen (28).

Discussion

These results have demonstrated that, for this colony of rats at least, the growth between the ages of 30 and 230 days on the highly purified Evans and Burr diet can equal that on a diet of mixed natural foods. The special supplements which permitted such optimum growth and

which, therefore, supplied the required appetite and utilization factors were cod liver oil and some source of vitamin B, such as yeast, yeast extract or tikitiki, and liver as a source of vitamin G. Optimum growth, which was associated with increased food consumption, appeared to depend upon the presence of the liver. The growth stimulating properties of liver have been observed by several investigators (29 to 32) since the earlier reports of Osborne and Mendel (4, 5).

Although these experiments were not originally intended to determine the role of fat in experimental diets, the results suggested that lard and particularly wheat germ oil exerted beneficial effects not shown by cod liver oil. The daily addition of 3 drops of wheat germ oil to the usual supplement of cod liver oil (Group 6) markedly improved the rate of growth, especially during the third period. Group 6 was the only group not receiving liver to exceed 300 gm. in weight. This group showed only fair growth during the first 60 days, better growth during the first 100 days and nearly optimum growth during the period in excess of 100 days. Group 13 which received wheat germ oil and liver extract showed growth nearest the optimum during the third period. Whether this effect on growth was due to vitamin E (33) is not known. However, the fact that 9 of 10 female rats on the experimental diet supplemented with yeast and cod liver oil produced litters not only once but also after a second mating (unpublished data) was considered as evidence of the presence of vitamin E in the diet, presumably in the cod liver oil (34). In view of the observation of Burr and Burr (19) on the effect of fat free diets on the water intake of rats it was interesting to note the apparently decreased water intake of the groups receiving lard and wheat germ oil (Table IV, Groups 4, 5 and 6). In general, the results were in agreement with those of McAmis, Anderson and Mendel (23) who concluded that small amounts of fat were beneficial in some unrecognized manner in low fat diets. They were also in accord with the findings of Burr and Burr (26) that fatty acids other than those in cod liver oil were required by the rat. It is possible that the effectiveness of the whole liver was due in part to the liver fat.

As has been stated, 0.5 gm. of whole yeast daily supplied water-soluble factors sufficient for fair growth. The failure of 1.0 gm. of yeast materially to better the growth obtained with 0.5 gm. was difficult to understand. Evans and his co-workers (35, 36) also observed that 1.0 gm. was not superior to 0.7 gm. of yeast although the addition of liver to the smaller quantity of yeast resulted in improved growth. These results with yeast emphasized the advantages of liver over yeast as a source of vitamin G.

Autoclaved yeast or autoclaved marmite have been the usual sources of G in experimental work ever since Goldberger demonstrated the effectiveness of this material in preventing pellagra. As was shown by Quinn (37) the relative amounts of B and G in different yeasts may show wide variations. Moreover, Williams, Waterman, and Gurin (38) have demonstrated that the vigorous treatment, particularly the alkaline autoclaving, required to destroy all of the B of yeast also destroyed a large part of the G. Liver, on the other hand, contains only a small amount of B and does not require autoclaving at pH 9 to give a product free from B. Undoubtedly, autoclaving of fresh liver does destroy some of the G but the resulting material has been found to be an excellent source of G. Guha (33) has also recognized the advantages of liver over yeast as a source of G.

Yeast has been widely used in nutrition investigations as a supplement which supplied the vitamin B complex and also as a source of material for experiments in which separation of active fractions has been attempted. The failure of some yeasts, at least, to permit more than fair growth demands serious consideration. Inasmuch as erroneous conclusions may be drawn from experiments in which comparisons are made with "standard or normal" growth curves representing only fair growth and not the actual growth capacity of the rats, it would appear imperative to demonstrate that optimum growth is possible on any chosen artificial diet before the diet is subjected to further examination.

There was no good evidence in these experiments for the existence of members of the B complex other than B and G. It was realized that the failure with yeast suggested an additional appetite factor in liver which was absent from yeast. However, 0.5 gm. of yeast did supply appetite and utilization factors sufficient for fair growth. The assumption of a new appetite factor in liver would still leave unexplained the failure of 1.0 gm. of yeast to accelerate growth through the increase in the appetite factors manifestly present in 0.5 gm. of yeast. The results suggested the alternative possibility that vitamin G might not be readily available in certain yeasts so that the amount of G actually furnished a rat might have very little relation to the quantity of yeast in the supplement. It is also possible that the larger quantity of yeast introduced some injurious substance which inhibited appetite.

Numerous reports have been made in recent years of the existence of additional members of the B complex (39 to 44) and it is not at all improbable that the occurrence of such factors may be definitely confirmed as a result of future work. Nevertheless, it is believed that certain of these

experiments may be justly criticised for acceptance of very mediocre growth as normal growth and others for failure to demonstrate that adequate amounts of G were actually available in the supplements. Chick and Roscoe (45) have also emphasized the need for caution in interpreting the results of investigations in which autoclaved yeast and particularly alkaline autoclaved yeast were used as a source of G. In this connection it is of interest to find that Hunt (46) has not been uniformly successful in the preparation of residues having the properties previously described and that Williams and Eddy (47) now suggest the possibility that the B_3 of Williams and Waterman (44) might be nothing more than an abundant supply of B.

ADDENDUM.—Since this paper was written several articles have appeared which offer additional support for the occurrence of a new growth factor or of growth factors. As far as we know, there is no evidence which denies the possibility or the probability of the existence of unrecognized members of the vitamin B complex. However, our own results have been of such a nature as to emphasize the need for caution in interpreting observations such as those of Halliday (48) because of the use of autoclaved yeast as a source of G and those of Stiebeling (49) because of the use of experimental animals receiving less than the optimum amount of G. The experiments of Mapson (50) demonstrated anew and in striking fashion the importance of liver as a source of growth factors. Nevertheless, it seems pertinent to raise the question as to whether or not the vitamin G requirement for the consumption and utilization of food for optimum growth has been fully supplied in these experiments.

SUMMARY

1. The rate of growth of rats on the highly purified Evans and Burr diet, properly supplemented, equaled that of rats on a diet of mixed natural foods.

2. Optimum growth on the purified diet was directly related to the increased consumption of food resulting from the presence of liver in the daily supplement.

3. Liver was found to be superior to yeast as a source of vitamin G.

4. The ratio of the water intake to the food consumption on the experimental diets was fairly constant and amounted to approximately 0.45 cc. per calorie.

5. Scaliness of the feet and scaliness and soreness of the tail were common on the experimental diet. These signs were less severe if liver or fats

other than cod liver oil were furnished in the daily supplements. This type of dermatitis was not related to vitamin G deficiency.

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STUDIES ON GROWTH

II. THE EFFECT OF VITAMINS B AND G ON THE CONSUMPTION AND UTILIZATION OF FOOD*

By

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IN A previous report (2) experiments on growing rats were presented which showed that the amount of the daily supplement of yeast, which was used as a source of the vitamin B complex, influenced the utilization of food. This effect of yeast was observed in experiments in which the food intake was restricted to a stated caloric level and was contrasted with the stimulation of appetite and the resulting acceleration of growth observed if liver was used as a supplement to the diet fed *ad libitum*.

Although the appetite of growing rats is affected by many dietary deficiencies, it has appeared to be most closely related to the vitamin B complex. This relationship was observed by Osborne and Mendel (3) and by Drummond (4) and has been investigated by Karr (5) and by Cowgill (6, 7). Their experiments were performed before the distinction between vitamins B and G had been definitely established. In more recent work Sherman and Sandels (8) found that the loss of appetite in rats was much more pronounced in the case of deprivation of B than of G and concluded that B exerted a specific effect upon appetite whereas the decreased food consumption in G avitaminosis was probably only the "usual concomitant of the weakness, lack of tone, and increasing torpidity of the experimental animals." Burack and Cowgill (9) also concluded that G was not concerned with the stimulation of appetite in dogs. Palmer and Kennedy (10) confirmed the observation of Graham and Griffith (2) that a supplement, such as liver, was mainly effective by a stimulation of appetite and not by an increase in the efficiency of utilization of food. They also demonstrated that there was little difference in the efficiency of utilization of an adequate synthetic diet and of a diet of natural foods.

* This paper and the previous one of this series (1) are based on data taken from the dissertation submitted by Claire E. Graham to the Graduate School of St. Louis University in partial fulfilment of the requirements for the degree of Doctor of Philosophy.

In the first paper of this series (1) it was shown that nearly optimum growth was possible on the properly supplemented Evans and Burr diet and that the optimum growth appeared to depend upon the presence of liver in the daily supplement. Inasmuch as the liver was rich in G and poor in B it was suggested that the effect of liver in increasing the food consumption was related to its content of G and that this vitamin might have some significance in the normal regulation of appetite. In the present series of experiments, in which over 600 rats were used, the effect of yeast and, more particularly, the effect of the vitamin B complex on appetite and the utilization of food have been investigated. The experiments were planned so that it would be possible to compare the appetite stimulants in supplements which resulted in mediocre growth with the appetite factors in supplements which had previously been shown (1) to permit optimum growth.

Experimental Procedure

Young male rats averaging 28 to 31 days of age and 49 to 51 gm. in weight were used. The Evans and Burr diet was employed and was supplemented in every case with 0.27 gm. of cod liver oil daily. The experimental period lasted 40 days and most of the groups were restricted to either 975 or 1365 calories during this period. The measured portion of the basal diet and the supplements were fed daily. The sources of the vitamin B complex were whole dried liver, whole dried baker's yeast, dried autoclaved hog liver, and tikitiki.[1] With the exception of one preparation of tikitiki the materials were the same as those used in the experiments reported in the first paper (1).

The charts show the gain in weight during the experimental period, the total calories consumed and the average caloric requirement per gram gain in weight. The latter value represents the efficiency of utilization (for growth) of food and is particularly significant in the comparison of groups having the same caloric intake.

Experimental Results

Groups 1 and 2 (Chart 1) were fed tikitiki and autoclaved liver respectively as the sole source of the vitamin B complex. In neither case was there any appreciable growth after the first ten days, although the period of survival was longer in the case of the rats fed the tikitiki. The food intake of those surviving the forty day period was approximately the same

[1] The same extract of "rice polish" was used in all of these experiments except those listed in Chart 3. The groups in Chart 3 received the tikitiki employed in the earlier experiments (1).

in both groups. It was concluded that the tikitiki and the autoclaved liver were practically free from vitamins G and B respectively.

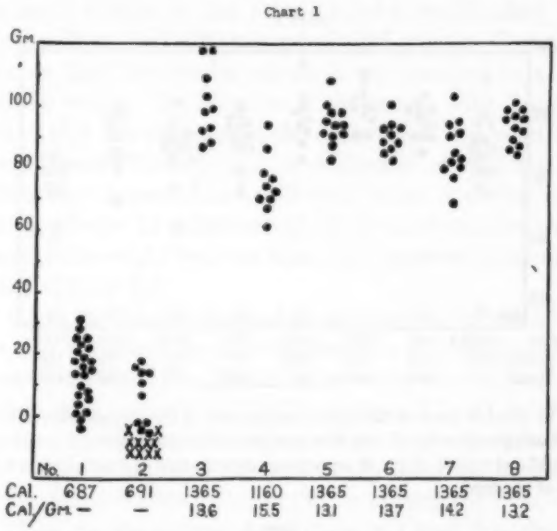

CHART 1. The gain in weight of young rats during a forty day experimental period on the Evans and Burr diet supplemented daily with cod liver oil and the following special supplements:

No. 1—0.25–0.50 cc. of tikitiki.
" 2—0.5–1.0 gm. of autoclaved liver.
" 3—1.0 gm. of whole liver.
" 4—0.15 gm. of yeast+0.85 gm. of autoclaved liver.
" 5—0.15 gm. of yeast+0.85 gm. of whole liver.
" 6—0.2 gm. of whole liver+0.1 cc. tikitiki.
" 7—1.0 gm. of yeast.
" 8—0.5 gm. of yeast+0.5 gm. of whole liver.

Groups 1 to 4 were fed *ad libitum* and Groups 5 to 8 were restricted to 1365 calories. Deaths during the experimental period are shown by the letter *X*.

Groups 3 and 4 were also fed *ad libitum* and were supplied supplements which permitted only mediocre growth. Since it was desired to investigate the utilization of food and the appetite under such conditions, groups were started and restricted to the food intake of Group 3, 1365 calories, during the experimental period. The maximum increase in weight of these rats ranged from 95 to 120 gm. Inasmuch as the same average gain in weight was obtained with a number of quite different supplements (3, 6, and 8, (Chart 1) 11, (Chart 2) 17 and 21 (Chart 3)) it was concluded that this represented the optimum utilization of the 1365 calories. These supple-

ments which permitted the same efficiency of utilization of food had varying effects upon the rate of growth if the basal diet was fed *ad libitum* (1).

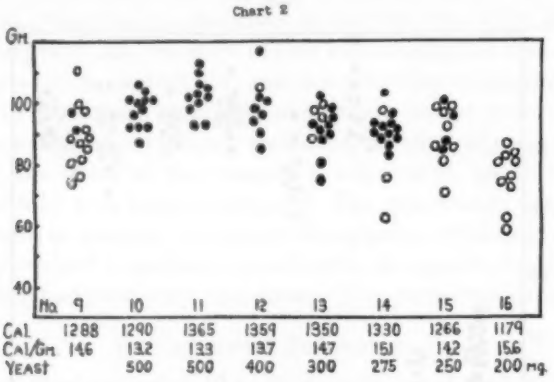

CHART 2. The effect of yeast on the gain in weight and on the consumption of food during the 40 day period. Each group except Group 10 was restricted to 1365 calories. Group 9 received 0.05–0.10 cc. of tikitiki and 0.10–0.15 gm. of autoclaved liver instead of yeast. Circles indicate failure to consume all of the food.

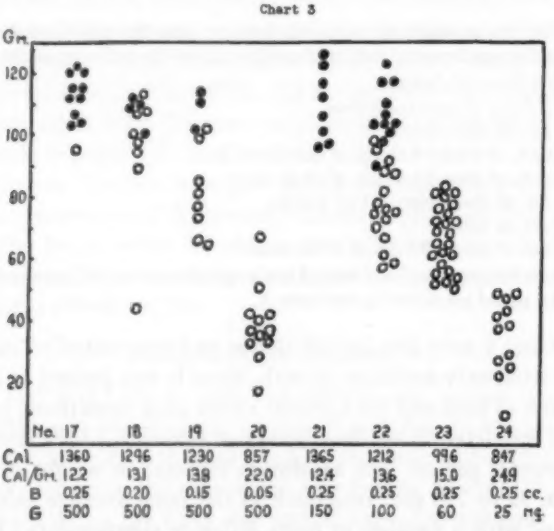

CHART 3. The effect of tikitiki (B) and of autoclaved liver (G) on the gain in weight and on the consumption and utilization of food during the 40 day period. Each group was restricted to 1365 calories. Circles indicate failure to consume all of the food.

Chart 2 shows the effect of yeast as a supplement. Optimum utilization occurred with 0.5 gm. of yeast daily (Group 11). In order to determine whether a small change in the caloric intake would affect the rate of growth, Group 10 was fed 1290 instead of 1365 calories. Comparison of 10 with 11 shows that the smaller caloric intake resulted in a slightly decreased gain in weight. Examination of the results with Groups 11 to 16 demonstrated that the decrease in the quantity of the yeast supplement progressively lowered the efficiency of utilization of food and the gain in weight. Utilization appeared to be affected before appetite since most of the rats in 12, 13 and 14 consumed all of the 1365 calories and yet their average increase in weight was less than that observed if optimum utilization occurred (Group 11).

Chart 3 shows the results obtained in experiments in which the vitamin B complex was furnished by varying amounts of tikitiki (B) and autoclaved liver (G). Groups 17 to 20 received an adequate supply of autoclaved liver and decreasing amounts of tikitiki. Groups 21 to 24 received an adequate supply of tikitiki and decreasing amounts of autoclaved liver. The resulting effect upon appetite was practically identical in the two series since appetite failed if either tikitiki (B) or autoclaved liver (G) was inadequate. These results differed from those in Chart 2 in that failure of appetite was not generally preceded by a marked decrease in the efficiency of utilization of food. A possible explanation of this difference was the fact that the lowering of the yeast supplement (Chart 2) decreased both B and G whereas in the groups in Chart 3 an adequate amount of either B or G was supplied in each case. Group 9 represents an attempt to adjust a daily supplement of tikitiki and autoclaved liver so that the minimum quantities required for the complete consumption of the food (1365 calories) would be supplied. The average food consumption of this group was 1288 calories, practically the same as that of 10 which was restricted to 1290 calories and which received a supplement permitting optimum utilization of food. The poorer utilization of food by 9 indicated that the efficiency of utilization could be decreased by inadequate supplements of autoclaved liver and tikitiki as well as by an inadequate supplement of yeast.

In view of the results which were obtained with a caloric intake of 1365 calories, another series of groups was started in which the caloric intake was restricted to 975 calories during the 40 day period. On this caloric level the maximum gain ranged from 60 to 85 gm. Approximately the same average gain resulted with several different supplements (29, 35 and 39) and it was assumed that this gain represented the optimum utilization of the 975 calories.

Chart 4 shows the results with yeast as the supplement. Optimum utilization occurred with 225 mg., or more, of yeast daily. With less than this amount the efficiency of utilization decreased first and the failure of appe-

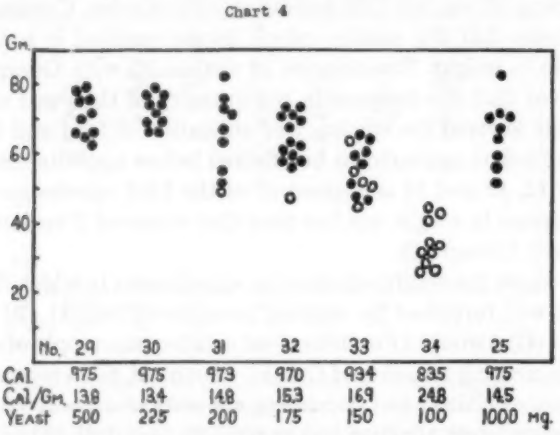

CHART 4. The effect of yeast on the gain in weight and on the consumption and utilization of food during the 40 day period. Each group was restricted to 975 calories. Circles indicate failure to consume all of the food.

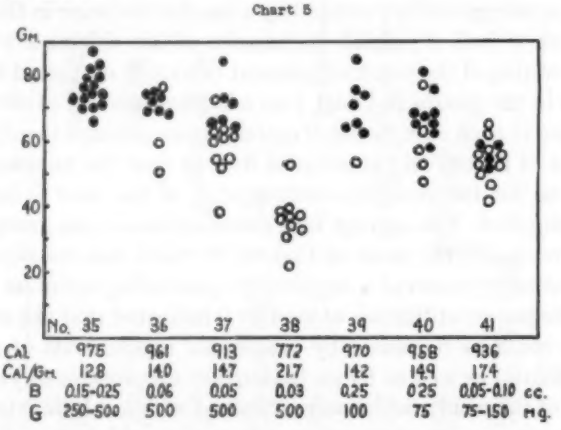

CHART 5. The effect of tikitiki (B) and of autoclaved liver (G) on the gain in weight and on the consumption and utilization of food during the 40 day period. Each group was restricted to 975 calories. Circles indicate failure to consume all of the food.

tite followed. These results agreed with those obtained on the higher caloric level (Chart 2). A supplement of 1.0 gm. of yeast daily (Group 25) was

not so satisfactory as the use of 0.225 to 0.5 gm. The rats in both 7 and 25 were slower in consuming the daily allowance of food and never appeared to be as hungry as those fed 0.5 gm. of yeast. Since even the 1365 caloric level represented considerably less food than would be consumed on a properly supplemented diet fed *ad libitum*, the results with a supplement of 1.0 gm. of yeast daily supported the conclusion stated in the first paper (1) that certain yeasts, at least, are decidedly inferior to combinations of tikitiki and autoclaved liver as a source of vitamins B and G.

Chart 6

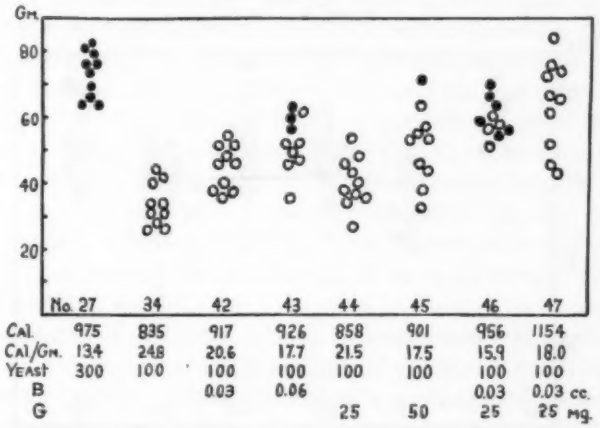

CHART 6. The effect of tikitiki (B) and of autoclaved liver (G) on the gain in weight and on the consumption and utilization of food during the 40 day period. Each group except Group 47 was restricted to 975 calories. Group 47 was restricted to 1365 calories. Circles indicate failure to consume all of the food.

Groups 35 to 38 (Chart 5) received an adequate supply of autoclaved liver and decreasing quantities of tikitiki. Groups 35, 39 and 40 show the effect of decreasing the autoclaved liver in supplements adequate with respect to tikitiki. The results were similar to those obtained with 1365 calories (Chart 3). As either B or G was lowered the gain in weight decreased. As in Chart 3 the principal effect appeared to be in the failure of the appetite. Group 41 represents the attempt to supply minimum quantities of B and G in order to duplicate, if possible, the results with yeast in which B and G were simultaneously reduced. The results were significant since comparison of 41 with 33 indicates that these two groups with practically the same caloric intake required approximately 17 calories per gram gain

in weight instead of the 13 calories required in optimum utilization. This result with 41 confirmed the previous observation that the efficiency of utilization of food is decreased if both B and G are inadequate.

Charts 6 and 7 present additional experiments in which the effects of tikitiki and of autoclaved liver on appetite were determined. The rats in Groups 34 and 42 to 47 received 100 mg. of yeast daily. This amount of yeast permitted very slow growth (34). The addition of 0.03 cc. and 0.06 cc. of tikitiki B daily slightly improved the appetite and growth but the same improvement resulted from supplementing the 100 mg. of yeast with

Chart 7

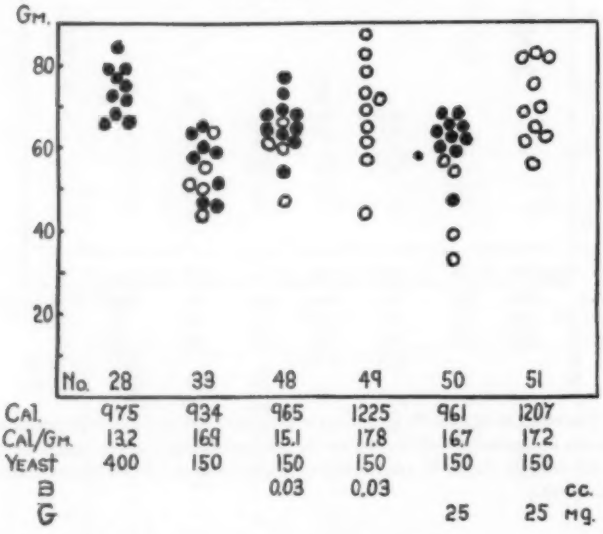

CHART 7. The effect of tikitiki (B) and of autoclaved liver (G) on the gain in weight and on the consumption and utilization of food during the 40 day period. Groups 28, 33, 48 and 50 were restricted to 975 calories. Groups 49 and 51 were restricted to 1365 calories. Circles indicate failure to consume all of the food.

25 and 50 mg. of autoclaved liver G. The results with 0.06 cc. of tikitiki and with 50 mg. of autoclaved liver respectively were inferior to the result obtained by supplying a combination of 0.03 cc. of tikitiki and 25 mg. of autoclaved liver. Chart 7 shows the effect of adding 0.03 cc. of tikitiki and 25 mg. of autoclaved liver to 150 mg. of yeast as supplements for groups on both the 975 and 1365 caloric levels. In neither case was the effect produced by tikitiki superior to that produced by autoclaved liver.

Discussion

Tikitiki and autoclaved liver, sources of B and G respectively, produced only moderate stimulation of appetite if added separately to supplements low in these two vitamins. The most marked increase in the consumption of food resulted from the addition of both tikitiki and autoclaved liver to the low B and G supplements. There was no evidence in these experiments that B was more necessary than G for the regulation of the appetite of young growing rats. The two vitamins not only appeared to be of equal importance in this respect but also seemed to depend upon the presence of each other for their activity. These data were obtained in experiments in which the appetite factors were concerned with maintenance and with slow growth. There was no apparent difference in the qualitative character of the appetite factors involved in the consumption of small amounts of food and in the optimum consumption of food (1) since slow growth or optimum growth could be produced at will by controlling the food consumption through variations in the amounts of tikitiki and autoclaved liver in the daily supplements. These experiments neither denied nor supported the assumption that the vitamin B complex consists of fractions other than B and G.

The efficiency of utilization of food by young rats was apparently unaffected by wide variations in the amounts of vitamins B and G in the daily supplements. Sources of these two vitamins producing only fair growth in *ad libitum* experiments in which appetite was a factor permitted optimum utilization if the food intake was restricted to a caloric level below that of the *ad libitum* experiments. However, utilization was affected if *both* B and G were simultaneously decreased to low levels. The fact that utilization was inferior was indicated by a smaller gain in weight even though all of the food was consumed. Utilization may also have been inferior on those supplements which were low in *either* B *or* G. In most of such experiments, however, appetite was decreased so that the rats could no longer be compared on the basis of equal caloric intake. It should be emphasized that these experiments were concerned with caloric levels which were considerably below the level of food intake in rats supplied an adequate diet *ad libitum*. The results were of interest in view of the observation of Palmer and Kennedy (10) that rats utilized adequate artificial diets as well as a mixture of natural food materials.

St. Julian and Heller (11) determined the coefficients of digestibility of protein, fat and carbohydrate on normal and deficient diets and found that the coefficients were unaffected even on diets low in B and G. This sug-

gested that the inferior utilization of food observed in our experiments on low B and G diets was connected with the metabolism of the ingested food. It was not possible to determine whether the decrease in efficiency was in the utilization of food for maintenance, for growth or for work. The importance of both vitamin B and vitamin G for the utilization and the consumption of food indicated that their metabolic functions might be closely related.

Summary

1. The influence of yeast and of combinations of tikitiki and autoclaved liver on the consumption and utilization of food in young rats have been determined in experiments in which the caloric intake was restricted to either 1365 or 975 calories during a 40 day experimental period.

2. Tikitiki and autoclaved liver, sources of vitamins B and G respectively, were found to be of equal importance as appetite stimulants.

3. The utilization of food (for growth) was found to be decreased on diets low in vitamin B and vitamin G.

4. It was concluded that vitamin G, as well as vitamin B, has an important part in the physiological mechanism controlling the consumption and the utilization of food.

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Editorial Review

THE EFFECT OF MUSCULAR EXERCISE ON THE METABOLISM OF ETHYL ALCOHOL

ALTHOUGH alcoholic beverages have been used by mankind for many centuries, it is surprising how little is absolutely known with regard to the utilization and metabolism of alcohol in the animal body. It is only within a decade or two that much advance has been made with regard to the fate of alcohol, particularly with regard to the concentrations of alcohol in the tissues and its disappearance from the tissues under various conditions. It is well known that in some countries, particularly in Europe, some groups of workers performing hard labor consume relatively large amounts of alcohol. It is therefore not only of academic interest, but of practical importance to know whether the metabolism of alcohol is accelerated by muscular exercise. If more alcohol is burned during muscular exercise than during rest and there is no serious impairment of efficiency, then alcohol is of value as a source of muscular energy, and it could be no longer contended that the energy for muscular activity was derived exclusively from any particular substance. If muscular exercise accelerates the combustion of alcohol, therapeutic use could be made of this fact when attempts are made to bring back an intoxicated individual to a normal condition or when one wishes to prevent the toxic and narcotic effects of large amounts of alcohol. If, on the other hand, there is no greater combustion of alcohol during muscular work than during the condition of rest, alcohol would occupy a unique place among the substances which can furnish appreciable amounts of energy in the animal body. Whatever may be the material, which is the immediate source of energy for muscular contraction, all classes of nutrients, protein and fat, as well as carbohydrates, are ultimately drawn upon to furnish directly or indirectly energy for muscular activity when there is an inadequate food supply. It is therefore of value both theoretically and practically to ascertain whether muscular activity accelerates the combustion and metabolism of alcohol.

The principal investigations on the effect of muscular activity on the metabolism of alcohol in men and animals are reviewed in this contribution.

Chauveau (7) made observations on the metabolism of a dog, varying 18 to 21 kilos, during a period of feeding, lasting over 389 days. The dog was made to run daily one or two hours in a rotary treadmill and his

respiratory exchange was measured at the same time. The respiratory exchange was also measured during periods of repose during the day, and during the periods of sleep at night. Shortly before the work began the dog was fed 500 grams of raw meat and 252 grams of sugar. From time to time there were substituted in this ration 48 grams of alcohol for 84 grams of sugar. The exercise varied from runs of 13.6 to 26.6 kilometers in the sugar experiments and from 9 to 21.1 kilometers in the experiments with alcohol, during approximately one to slightly over two hours. The average respiratory quotient in the four experiments without alcohol during the exercise periods was 0.963, whereas in the seven experiments in which alcohol was substituted, the respiratory quotient was 0.922. Chauveau calculated that the respiratory quotient should be 0.763 with 168 grams of sugar and 48 grams of alcohol if the respiratory quotient were 0.963 with 252 grams of sugar. The quotients found during the periods of repose were 0.904 and 0.871 as compared with a theoretical quotient of 0.716, when alcohol was substituted. The quotient for the total day was 0.921 on the days without alcohol and 0.885 instead of the theoretical 0.730 on the alcohol days. According to the author the enormous differences between the results found and those theoretically calculated show that when the organism is saturated with alcohol it is not able to utilize alcohol as a source of potential energy, either for the execution of the total physiological processes in the state of repose, or for the performance of work during muscular exercise.

The conclusions of Chauveau have been criticised by v. Hoogenhuyze and J. Nieuwenhuyse (22) for considering only the respiratory quotient and ignoring the lower carbon dioxide elimination and oxygen absorption during work on days with alcohol. They also consider the dose too disproportional (authors' word) to the body weight to be of practical value.

Atwater and Benedict (1) made several series of 24-hour experiments with humans in a respiration calorimeter. The balances of nitrogen, carbon, and energy were determined for each day. The available energy of the intake varied from 3502 to 3623 calories for one subject, and 3216 to 3276 calories for the other. The subjects worked 4 hours each morning and afternoon daily on a bicycle ergometer. In the first series the work equalled 158 to 211 calories, in the second 237 to 270 calories, and in the third series 127 to 277 calories per day. In the first series the diet on 4 days contained, beside protein, 129 grams of fat and 484.6 grams of carbohydrate, and then on 4 days the diet was 158.5 grams of fat, 296.1 grams of carbohydrate and 72.4 grams of alcohol. In the second series of experiments there were 9 days, 3 days with 106.0 grams of fat, 470.7 grams of carbohydrate, 3 days

with 104.2 grams of fat, 340.9 grams of carbohydrate, and 72.0 grams of alcohol, and then 3 days with 160.8 grams of fat and 342.7 grams of carbohydrate. The last series consisted of 9 days with two 3-day periods of fat and carbohydrate and one 3-day period in which 72 grams of alcohol replaced 122.9 grams of carbohydrate. The energy balances were negative on all but one of the days. In the 5 groups of days without alcohol the balances varied on the average from −329 to −435 calories. In the 3 groups with 72 grams of alcohol the balances were −313, −262, and −405 calories.

The authors drew the following conclusion: "That a part of the potential energy of the alcohol was transformed into the kinetic energy of muscular work these experiments do not prove, though they make it highly probable. They imply, that, so far as the utilization of the total energy of the diet was concerned, there was a slight advantage in economy in favor of the ordinary diet as compared with the alcohol diet, especially when the subjects were at hard muscular work, but the difference was inside the limits of experimental error and too small to be of practical consequence. On the average it was less than 1 per cent of the total energy and hardly reached 5 per cent of the energy of the alcohol. From this it follows that the energy of the alcohol was utilized nearly if not quite as well as that of the fats, sugar, and starch which it replaced."

This conclusion has been repeatedly cited in part or wholly by most of the investigators who have since studied in this field.

Grehant (10) determined by the Nicloux method the rate of disappearance of alcohol from the blood in dogs before and after the carrying out of muscular exercise. A dog was given by stomach tube 25 cc. of 10 per cent alcohol per kilo and was made to run in a rotary wheel 5.65 kilometers during one hour. Samples of blood were taken from the jugular vein one and two hours before the beginning of work and again at hourly intervals after the work was finished. The introduction of the alcohol took place at 11 to 11:15 a.m. and the first sample of blood was taken at 1:45 p.m. Between 3:45 and 4:45 p.m. the dog exercised and the drop in content of alcohol per 100 cubic centimeters was 0.05 cc. during the work as compared with 0.02 cc. and 0.04 cc. before the work and 0.035 after the work. In another experiment a dog was given 25 cc. of 10 per cent alcohol per kilo at 8 to 8:20 a.m. Samples were taken at hourly intervals beginning 5 hours later. The fall in blood alcohol was 0.029 cc. and 0.0285 cc. before the work, 0.055 during the work, and after the work ceased 0.009. The author concluded that muscular exercise favored the disappearance of alcohol but less than one supposed from *a priori* reasoning.

Durig (8) conducted observations on himself as to the influence of alcohol upon the respiratory exchange and performance of muscular work at high altitudes. The series consisted of 12 trips of mountain climbing with a load of 18 to 19 kilograms at an altitude of 2430 meters and in each of the 12 there were 4 successive periods of measurements. Four of the days with 16 periods were after the ingestion of alcohol in the morning and the alcohol days alternated with days on which no alcohol was taken. On the alcohol days the subject took 30 cc. of alcohol diluted with 125 to 250 cc. of water and sweetened with sugar at breakfast instead of the tea taken on other days. After camp duties were performed, which required one-quarter to one-half hour after taking alcohol, there was a walk of one hour to the place of the experiment. Then followed the four periods of work at approximately 800 kilogrammeters per minute. On the non-alcohol days the respiratory quotients in all 4 groups fell from the first period to a somewhat lower value by the fourth period: from 0.87 to 0.83, 0.86 to 0.84, 0.78 to 0.75, 0.81 to 0.76. On the alcohol days, on the contrary, the first quotients on two days were lower than any of the succeeding quotients, that is, there was a rise from 0.75 to 0.81 and from 0.82 to 0.86. On the other 2 days there was a change from 0.85 to 0.81 and no definite change in the fourth series. The author reasoned that if the alcohol were not burned in the preliminary walk, the respiratory quotient would have been lower during the experimental periods themselves because the alcohol would be burned at that time, and should be lower than the values on the non-alcohol days. Because of the fact that the quotient rose instead of falling on two days, he reasoned that the greater part of the alcohol was burned during the preliminary hour of walking, that gradually less alcohol was available for the performance of muscular work because of the disappearance of alcohol, and therefore the respiratory quotient would gradually rise during the four successive periods of work. In other words, the alcohol had practically disappeared during the preliminary hour of walking when no measurements were made, and, therefore, was utilized to supply part of the energy for the performance of muscular work.

These experiments have been cited by many authors, particularly with reference to another phase of the study, that is, the efficiency, but it is evident that they furnish but very little proof of any actual combustion of alcohol during muscular work. The evidence and reasoning is wholly indirect and in the light of today's knowledge it is questionable whether the alcohol would have been absorbed so fast and so completely during one and one-half hours that it would have been burned by the time the first period began. There is also the known effect of food which would ob-

scure the study with reference to the availability of alcohol for work. It is well known that the presence of food delays the absorption of alcohol so that it never rises to the same degree of concentration in the blood as it does when taken on an empty stomach. In addition to the sugar there was also ingested bread, so that there was food enough in the stomach to lower the absorption rate of alcohol.

Völtz and Baudrexel (24) determined the alcohol eliminated in the respiration and urine of a dog weighing 10 to 11 kilograms after the ingestion, at the beginning of the experimental day, of 1.7 to 1.9 cc. of alcohol per kilogram, i.e., 19.44 and 20 cc. of a 9.73 per cent solution by volume. In one series of observations the dog rested the entire day and the expired air was collected for 3 hours from a mask attached to the animal. The remainder of the day the dog was in a respiration chamber. In a second series there were 5 minutes of running, with 5 minutes pauses, with a total of 3 hours running and rest, during which the expired air was collected from a mask. The animal was in a respiration chamber the remainder of the day. The total run averaged 8.43 kilometers. In a third series, the dog ran 10 minutes, rested 5 minutes, for a total period of 3 hours with a run of 11.4 kilometers. In a fourth series the amount of exercise was the same, but the exercise did not begin until after a lapse of 3 hours after the alcohol ingestion. The per cent of the total ingested alcohol eliminated in the respiration in 3 hours averaged 0.75 per cent in the first series of four rest experiments; 4.27 per cent of the total in the series with 1½ hours' total exercise; 5.66 per cent of the total ingested alcohol in the series with 2 hours' work; and 3.68 per cent in the series with 2 hours of exercise when the exercise began 3 hours after the ingestion of alcohol. The total amount of alcohol eliminated in the respiration in 22 to 23½ hours in the same four series of experiments was as follows: first series, 2.02 per cent of the alcohol ingested; second series, 5.07 per cent; third series, 6.68 per cent; and fourth series, 4.82 per cent of the amount of alcohol ingested. Thus, exercise in a dog amounting to a run of over 11 kilometers in 2 hours' time resulted in a marked increase in the elimination of alcohol in the breath as compared to the amount eliminated when the animal was at rest. The reviewer considers that the possible part played by the elimination of alcohol in the breath during exercise, in decreasing the alcohol concentration in the body, must not be neglected in judging of the effect of exercise on the metabolism of alcohol.

Krieger (14) carried on a series of observations on himself with respect to the nitrogen balance as affected by muscular work with and without the addition of alcohol. The diet was so arranged that it was insufficient to

cover the daily needs with respect to the energy requirement and there would thus be always a nitrogen loss, particularly on the days on which there was muscular activity. The diet contained approximately 17.2 grams of nitrogen, 74 grams of fat, 177 grams of carbohydrate, with a total energy content of 1855 calories per day. There were five experimental periods. The first was a preliminary period of 5 days during which the diet was taken and the ordinary light activity of laboratory work carried on. This was followed by a period of 9 days without alcohol during which the subject rode a bicycle 50 kilometers a day. An intermediate period of 4 days followed in which the diet and muscular activity were the same as in the preliminary period. There was then a period of 9 days during which there was added to the daily diet 1600 cc. of wine containing 6.56 per cent of alcohol and the work was similar to the previous 9-day period. This period was followed by a period of 5 days which was similar to the preliminary period. It was estimated that the energy required to cover the work performed was equal to 729 calories and that this could be supplied by 104.1 grams of alcohol. The wine actually contained 105 grams. It was taken in 4 portions during the day.

There were average negative nitrogen balances in all of the periods with the exception of the work period of 9 days in which alcohol was taken in addition to the diet. Specifically, in the work period without alcohol, there was an average negative nitrogen balance of 1.86 grams per day. On the contrary, in the period with alcohol and work there was an average positive balance of 0.12 gram of nitrogen per day.

The author concluded that the alcohol became available for the performance of muscular work, as shown by the fact that the negative nitrogen balance without alcohol was changed to a plus nitrogen balance with alcohol. However, the average nitrogen intake was 16.36 grams per day in the work period without alcohol and 17.08 grams per day with alcohol, so that part of the shift from the negative balance to a positive balance may have been due to the slight increase in the food intake. This would render questionable whether the alcohol actually furnished energy for the performance of muscular work. It is doubtful whether experiments of this sort can be utilized to demonstrate the availability of alcohol for the performance of muscular work.

v. Hoogenhuyze and Nieuwenhuyse (22) determined the respiratory exchange by means of the Zuntz-Geppert apparatus before and after taking alcohol on rest days and on days during which muscular work was performed. The procedure was as follows: At 9 a.m. the subject reclined in an easy chair. A determination of the respiratory exchange was made and the

subject took 60 cc. of 90 per cent alcohol diluted with 90 cc. of water. After 10 minutes he breathed through the valves for another 10 minutes and then samples of air were collected for 6 minutes. An hour later another test was made and likewise at 3 p.m.

On the work days the program was as follows: At 9 a.m. the resting metabolism was determined with the subject sitting on a bicycle ergometer. He then pedalled for 20 minutes at the rate of 500 kilogrammeters per minute. Then, on the alcohol days, the same amount of alcohol was given as previously and immediately the pedalling was begun at the rate of 380 kilogrammeters per minute. The subject breathed for 15 minutes freely and then for 5 minutes through the valves and a sample was then taken. An hour later the exercise was again resumed and the expired air was again collected for analysis. The same process was repeated at 3 p.m. Apparently the subjects had both breakfast and lunch on the experimental days.

The average respiratory quotient of one subject was 0.87 on the resting day before taking the alcohol, 0.78 twenty minutes after the alcohol, and 0.75 one hour later. At 3 p.m., it was 0.86. The other subject had an average respiratory quotient of 0.91 before the alcohol and 0.72, 0.74, 0.78 after taking alcohol. On the non-alcohol days the drop in respiratory quotient either did not take place or was not nearly so marked.

On the days with muscular work, the average respiratory quotient with one subject was 0.82 during rest, 0.88 during work 20 minutes after alcohol, 0.84 at one hour, and 0.89 at 3 p.m. The other subject had a change from 0.85 during rest to 0.81, 0.78, with a rise to 0.85 at 3 p.m. Both the carbon dioxide and the oxygen values were lower after taking alcohol than in the corresponding periods without alcohol on the non-alcohol days.

Their conclusion was that alcohol directly or indirectly, not only produced energy for muscular exercise, but that the muscular exercise was performed more economically immediately after the taking of alcohol.

Mellanby (16) determined the alcohol in the blood of dogs at rest and before and after muscular activity during the course of 6 hours after the ingestion of 30 cc. and 50 cc. of alcohol. In the resting experiments the dogs were confined to a small cage and in the exercise experiments the animals were encouraged to run as vigorously as possible about in the open. When the larger quantities were given, the dogs ran behind a bicycle, but not to exhaustion, which is brought on easily in animals under the influence of alcohol. When the dogs received 4.1 cc. of alcohol per kilo, the results indicated that the alcohol was but slowly consumed, and that there was but little difference between the resting and active states. When the dogs were given 30 cc. of alcohol, or 2.5 cc. per kilogram of weight, the exercise in-

creased the rate of disappearance so that after exercise there was approximately 4 cc. less alcohol in the body than in the rest experiments, assuming that the concentration of alcohol in the blood was a measure of the total alcohol in the body. The results were even more pronounced when 20 cc., or 2.0 cc. per kilogram, were given. Roughly, the animal resting had 6 cc. more of alcohol in the body at the end of 6 hours than the animal working.

In another observation the dog ran about except for a rest of one hour after the alcohol had been ingested. Toward the end of the experiment there was also another rest period. During the rest periods the fall in concentration of the alcohol in the blood was less than during the work periods. As a possible explanation of the difference between the results with the higher concentrations and the lower concentrations, Mellanby suggested that the dogs were capable of doing more work at the lower concentrations because they were not under so great a toxic influence of the drug. They were more active, although it must be pointed out that even with the larger doses, notwithstanding greater fatigue, the dogs probably covered about 8 to 10 miles in the experimental period.

His general conclusion was that alcohol combustion underwent a more rapid rate during exercise than during rest when alcohol is present in low concentrations, but that at higher concentrations alcohol is oxidized at the same rate in the resting and active animal. That is, the greater the toxic action of the alcohol, the more restricted is the increase in the rate of combustion by exercise and the closer are the rates of combustion of alcohol in the active and resting states.

Viale and Gianturco (23) made observations on the physiological effects of alcohol at an altitude of 2900 meters and in Turin and Naples. They measured pulse, respiration, vital capacity, volume of expired air, and respiratory exchange before and after the ingestion of alcohol. Alcohol was taken as diluted brandy containing 28 cubic centimeters of alcohol. The respiratory exchange was measured by means of the Douglas bag method before and at 30-minute intervals after ingestion of alcohol. The work was climbing and descending 100 meters. They came to the conclusion that small doses of alcohol were useful because of their moderating influence on heart strain and on the respiratory mechanism, and that alcohol could also be used for the production of work and heat in the place of other materials, and that this was particularly evident on high mountains.

Miles (18, 19) determined the alcohol in the blood and urine of a human subject at intervals during the course of nearly 4 hours. In one experiment the subject exercised on a bicycle ergometer at the rate of 1.3 calories

external work per minute in 15-minute periods that alternated with periods of psychological tests. Three samples of blood were taken after the ingestion of 27.5 grams of alcohol in 1000 cc. of water and grape juice. These samples were drawn immediately after the work period while the subject was still sitting on the bicycle ergometer and urines were collected as soon as possible thereafter. On another day a similar experiment was carried out, but the subject rested the entire time with the exception of the activity which might have to do with the taking of the samples of urine and blood.

In the experiment with work the maximum concentration of alcohol in the urine was 0.42 milligram per cc. at 80 minutes after the ingestion and the maximum alcohol content of the blood was 0.20 milligram at approximately the same period of time. After 170 minutes had elapsed after the ingestion of the alcohol, the urine contained 0.33 milligram of alcohol per cc. and the blood 0.13 milligram per cc. In the experiment in which no muscular work was performed, at 80 to 82 minutes after ingestion the urine contained 0.44 milligram and the blood 0.28 milligram of alcohol per cc. and at 170 minutes after ingestion the urine had 0.29 milligram and the blood 0.13. The blood shows practically no greater change in alcohol concentration on the day with exercise than on the day at rest. The urine has almost identical values in both experiments, so that in Miles' experiments there is no evidence that the performance of muscular work after the taking of alcohol affects the concentration of alcohol in either blood or urine.

Sommerkamp (20) determined the course of the nitrogen excretion in two hour periods from 7 a.m. to 9 p.m. under conditions of fasting and rest and compared it with the course of the nitrogen elimination on other fasting days in which muscular exercise was performed between 11 a.m. and 1 p.m. The exercise consisted of either 25 kilometers of bicycling or 5 kilometers of rowing during two hours. On two days the same amount and kind of work was performed, but at the beginning of the work, 70 grams of dextrose in 200 cc. of water were taken in one case, and 100 grams of dextrose in the other. Similarly, with the same kind and amount of work, 50 cc. of alcohol diluted to 200 cc. in volume were ingested on one day, and 76 cc. of 90 per cent alcohol diluted to 200 cc. were ingested on another.

The nitrogen excretion on the 5 days in which muscular work was performed without food fell from an average of 1.29 grams per 2 hours before work to an average of 0.76 gram per 2 hours at the end of the work period. The excretion on the 2 days with the dextrose fell from an average of 1.19

grams per 2 hours before work to an average of 0.81 gram per 2 hours at the end of the work period. Following the period of work on the days on which no sugar or alcohol was taken, there was a rise after the work period to higher levels, ending at the period from 5 to 7 p.m. between 0.68 and 1.13 grams per two hours. However, on the days with dextrose during the interval from 5 to 7 p.m., the nitrogen elimination for 2 hours was 0.69 and 0.80 gram. Thus, the nitrogen elimination did not rise on the day on which dextrose was given after the work as it did on the day when no nutrients were ingested.

On the two days with alcohol, the nitrogen elimination in the period from 7 to 9 a.m. averaged 1.28 grams. During the period from 11 a.m. to 1 p.m. the nitrogen elimination was 1.05 grams. Thus, during the work period on the alcohol days there was a greater elimination of nitrogen than on any other days, but following the period of work there was a marked drop for the next two periods in the nitrogen elimination, in all cases below 0.70 per 2 hours, and in the periods from 5 to 7 p.m. there was as low a value as 0.42. Thus, the drop in the nitrogen elimination on the alcohol days was even greater than on the days on which dextrose was given, and also greater than on the days on which no nutrients were ingested and work was performed.

The author concludes from his experiments that alcohol not only spares carbohydrate and fat, but that its energy is directly available for the performance of muscular work and that this is the first proof for it, which might be considered free from criticism.

Brechmann (2) made a study of the respiratory exchange with respect to the availability of alcohol for the supply of energy for muscular work. The experiments were carried out by the author on himself and the respiratory exchange was measured by means of the Zuntz-Geppert apparatus. The work was performed on a hand friction ergometer. The procedure of the experiment was as follows: The subject took breakfast which consisted mostly of carbohydrate. At 12 o'clock noon the respiratory quotient was determined. Then between 1:00 and 1:30 p.m. the midday meal was taken, consisting principally of carbohydrate. This was followed immediately by the ingestion of 60 grams of fructose. At 3:25 p.m., 40 grams of fructose were taken. The respiratory quotients were measured in the rest experiments at 2:15, 3:15, 4:00, and 5:30 p.m. On the days on which alcohol was given, 30 grams were taken at 2:45 p.m.

In the rest experiments without alcohol, the respiratory quotients were all practically at unity or over, with the exception of the first quotient on one day which was 0.71. On the rest days on which alcohol was taken, the

quotient at 3:10 p.m. in one case was 0.86, a drop from 1.04, and in the other case, 0.71, a drop from 0.99.

On the work days the work was performed from 11:45 to 12 noon, with a total amount of 3900 kilogrammeters. On the day without alcohol there was a rise from the respiratory quotient of 1.09 at 12 noon to 1.23 at 2:15 and 3:20 p.m. On the alcohol day, however, the quotient at 3:15 p.m. was 0.88, a drop from 1.16 on the measurement preceding at 2:15. Subsequently two series were carried out in which work was performed to the extent of 3600 kilogrammeters immediately preceding each measurement of the respiratory quotient. On the day without alcohol there was practically no change in the respiratory quotients throughout the series. On the day with alcohol the quotient at 2:25 p.m. immediately after work before taking alcohol was 1.10, whereas, at 3:30 p.m. it was 0.87 after the alcohol was taken.

The author came to the conclusion that the alcohol served as a source of energy for the performance of muscular work. However, the experiments are not conclusive to the reviewer because of the fact that the respiratory exchange was not measured during the work itself. There is no indication that the alcohol was utilized during the period of work. It is questionable whether a plan of investigation such as was carried out by Brechmann can contribute much to the question of the utilization of alcohol for the performance of muscular work.

Brechmann (3) made a further series of observations on the effect of ingestion of alcohol on the respiratory exchange during work and during rest. The routine of the experiments was as follows: The subject had breakfast at 9 a.m., consisting of coffee, milk and sugar, and zwieback. At 11:15 a.m. the principal meal of the day, consisting of noodles with sugar, potatoes, and bananas, was taken. The measurements began at 12 noon and lasted six hours. At 1:15 and 3:30 p.m. 60 grams of fructose were ingested. On a second day the same procedure was used, but at 2:25 and 3:25 p.m. 28 grams of alcohol in the form of 60 cc. of brandy were ingested. The work periods lasted as a rule 20 minutes with approximately 9000 kilogrammeters being performed. The measurements of the respiratory exchange during the work began after the subject had worked 10 to 15 minutes and usually it was measured for 5 minutes.

In all of the work periods, both on the days with and without alcohol, the respiratory quotients were lower than in the rest periods immediately preceding. For example, on the first day without alcohol, the respiratory quotient at 2:45 p.m. at rest was 0.97, at 3:20 p.m. during work it was 0.90. On the alcohol days the change at the same period of time was from

0.94 to 0.78 and at 4:15 and 4:40 p.m. the change was from 0.80 at rest to 0.74 during work. A similar series with dextrose showed always the same phenomenon, that is, a fall in respiratory quotient during the work period that was greater on the day with alcohol than on the day without alcohol.

The author explained the lowering of the respiratory quotient during work on the days without alcohol as due to a carbon dioxide retention in the tissues. The author concludes that the alcohol was available for the performance of muscular work and that more of it is burned during work than during rest. The results in this investigation are so at variance with other investigations with respect to the fall in respiratory quotients during the work periods that the reviewer is inclined to question their significance. In addition the first respiratory quotients measured at 12 noon seem low for so high a carbohydrate diet as was taken at 9:00 and 11:15 a.m. These respiratory quotients ranged on 6 days from 0.76 to 0.97. Excluding the highest value, the range is at the upper limit to 0.86. These quotients are therefore no higher than one would obtain frequently with a subject who has fasted for 12 hours, and these facts together with the invariable lowering of the respiratory quotient during work periods render the results doubtful. The rate of muscular work was about 450 kilogrammeters per minute and it has been our experience as well as that of others that the respiratory quotient during the first 20 minutes at this rate of work is raised from the resting value instead of lowered.

Galamini (9) determined by a modification of the method of Widmark every half hour the alcohol content of the blood at rest and during muscular work when alcohol was given at the rate of 0.5 cc. per kilo and diluted with an equal part of water. The alcohol solution was taken on an empty stomach just before beginning the work, which consisted of ascending and descending stairs, or in walking at a rapid pace in the streets of Rome. The alcohol in the blood at rest was as follows: 0.53 at 70 minutes, 0.55 at 25 minutes, 0.56 at 15 minutes and 0.51 milligram per cc. of blood at 45 minutes. When alcohol was ingested at the beginning of work, the alcohol in the blood was 0.26 in 60 minutes, 0.24 in 45 minutes and 0.32 milligram per cc. in 65 minutes. In spite of the lower figures during muscular work, the author concluded that because the glucose content of the blood after the ingestion of cane sugar was not appreciably altered by the ingestion simultaneously of alcohol, it was a question whether the alcohol was used during muscular work. He found practically the same increase in the sugar of the blood during muscular work whether cane sugar was given with or without alcohol and concluded that further experiments were necessary

with measurements of the respiratory exchange in order to settle the question.

Carpenter (4) exposed hens to alcohol vapor in a chamber for periods of 2 to 27½ hours. The animals were confined in a wire cage which was supported in such a manner that records of the activity or lack of activity were made upon a long paper kymograph during the entire time of exposure. The largest group was exposed from 15½ to 16½ hours individually. It was found that at the extreme limits of concentration of alcohol in the tissues, namely, from 0.29 to 2.27 milligrams of alcohol per gram of tissue, there was an inverse relationship between the activity and the concentration of alcohol in the whole body, that is, the more active the animals, the less was the alcohol in the body at the end of the exposure. This was most marked at the extremes and did not follow necessarily in the order of graded activity. In order to produce artificial activity, the hens were stimulated by periodic shocks from an induction coil. These hens, however, showed about the same range of alcohol concentrations as the group in which the hens were free to respond according to their own condition. In general, the most active hens in the group were lowest in concentration of alcohol, whereas the less active had the highest amount at the end of the exposure. With a group of 6 hens exposed repeatedly to alcohol vapor, a direct rather than inverse relation seemed to exist between activity and alcohol concentration in the tissues.

By the method of exposure it would seem as though the greater the activity the greater would be the absorption of alcohol, because activity resulted in an increased respiration, which automatically would bring a greater amount of alcohol into the lungs to be absorbed from the inspired air. These experiments as a whole indicate that the effect of activity was to prevent the accumulation of high concentrations of alcohol. The evidence is entirely indirect and unfortunately no comparisons were made between the total metabolism during exposure and the concentration of alcohol in the tissues.

Terroine and Bonnet (21) made a study on the effect of the ingestion of alcohol on the heat production of men, pigeons, and rabbits, in the conditions of thermic neutrality with men and in the conditions of both thermic neutrality and at temperatures at which an extra heat production was required on the part of the animals. The men received 400 to 600 cc. of white wine containing 10.8 per cent of alcohol. Their basal metabolism was first measured in 15-minute periods by means of a mask and a Tissot spirometer. After the ingestion of the alcohol, the respiratory exchange was measured for over 8 hours. From the respiratory exchange was computed

the extra heat which was produced and this was compared with the potential energy of the alcohol. In one case, the extra heat equalled 94.3 per cent, in the other case, 92.3 per cent of the potential energy of the alcohol ingested.

With the rabbits, the alcohol was introduced intraperitoneally. With the pigeons it was introduced by intramuscular injection or by the mouth. The heat production of the pigeons at 29° was 6.5 calories per kilogram per hour before ingestion. After ingestion it was 8.4 and 8.6 and the total extra heat amounted to 94 and 95.5 per cent of the potential energy of the alcohol actually absorbed by the animals. At 12° the heat production of the pigeons before ingestion averaged approximately 8.6 calories and after ingestion the heat production varied from 8.3 to 8.9 calories per hour per kilogram. Thus, there was practically no change in heat production at 12° as the result of ingestion of alcohol. In 2 experiments with rabbits the extra heat production was equal to 96 and 97.4 per cent of the calorific value of the alcohol absorbed.

The authors concluded that at thermic neutrality the energy from alcohol can not take the place of energy required for synthesis or growth, or for external muscular work, that is, that it burns without any profit to the organism. On the contrary, when there is a cause for extra heat production as at low temperatures, alcohol can furnish heat. Therefore, the nutritive value of alcohol consists in conversion into heat only and alcohol can not be utilized in cellular transformations of energy or in the performance of muscular work.

The results of these experiments are quite at variance with the results found by other workers with respect to the extra heat production as the result of the ingestion of alcohol. There were astounding increases in heat production in individual periods with the human subjects, for example, from 17.13 calories per 15 minutes before alcohol ingestion to 35.75 calories in 15 minutes, an increase of over 100 per cent. The study, however, is highly suggestive with regard to a method of approach to a solution of the question of the nutritive value of alcohol.

Cassinis and Bracaloni (6) determined the alcohol in the blood and the urine of men at intervals after giving 0.5 cubic centimeter of alcohol per kilo, actually from 30 to 35 cc., diluted with a double volume of water. A series of measurements with the subjects at rest showed maximum concentrations of alcohol in the blood of 0.76 milligram per cubic centimeter at 41 minutes in one subject, 0.565 at 50 minutes and 0.73 at 40 minutes in others. With a run of 3600 meters (presumably on a level grade) at the 29th to 55th minute after ingestion, the maximum concentration of al-

cohol was 0.42 milligram at 48 minutes. With a run of 3600 meters from the first to the 25th minute, the maximum concentration of alcohol was 0.35 at 28 minutes, but subsequently at the 86th minute it was 0.48 milligram per cc. of blood. With a march of 9600 meters from the 17th to the 110th minute, the maximum concentration was 0.466 at 65 minutes. With stationary rowing from the 13th to the 63rd minute, there was a maximum concentration of 0.559 at 30 minutes, and subsequently a fall to a value of 0.29 at 115 minutes. Thus, muscular work in these cases produced a noticeable effect in preventing a rise in the percentage of alcohol in the blood to the same extent that occurred during rest.

Meyer (17) determined by the Douglas bag method the respiratory exchange of a man of 64 kilograms accustomed to large quantities of alcohol. This subject took 240 cc. of absolute alcohol in the form of beer and whiskey and food, consisting of 2 sausages on buttered bread in the course of two hours and was not affected enough to prevent the performance of muscular work on a bicycle ergometer. The respiratory exchange was measured after 5, 20, 40, and 50 minutes of work at 700 kilogrammeters per minute. One series of experiments was immediately after taking the alcohol, another followed two hours after the taking of the alcohol, and a third 12 hours after the ingestion of the alcohol. The main object of the study was to determine the efficiency but the respiratory quotients are given as averages for the measurements of one hour. With alcohol immediately preceding the work, the average respiratory quotient was 0.88 in four experiments. In 8 control experiments it was 0.868. When the alcohol was taken 2 hours before work, the average respiratory quotient during the work was 0.89. When the alcohol was taken 12 hours before the work, the average respiratory quotient during the work was 0.853 as compared with 0.87 in the control experiments. The author pointed out that the respiratory quotients show that the combustion of the alcohol was extremely slow and that with this subject who was accustomed to large amounts of alcohol, the rapidity of burning was no greater, and in fact, not so great as has been found in earlier studies with subjects unaccustomed to alcohol.

These experiments seem extraordinary because of the ability of a man to carry on muscular work after the consumption of 240 cc. of alcohol (3.75 cc. per kilogram) and the apparent lack of combustion of alcohol during the work. The first series of experiments was undoubtedly too soon after the consumption of the alcohol in order for its full effect to have taken place, but two hours after the ingestion of alcohol one would expect that enough had been absorbed to furnish an appreciable amount for the metab-

olism for muscular work. Similarly, at the end of 12 hours with this amount of alcohol, there must have remained in the body a considerable amount so that it was still available for the performance of muscular work. It is difficult to conceive of the disappearance and combustion of this amount of alcohol in the body without affecting the respiratory quotients.

Hitchcock and Kraft (13) in a preliminary report at the annual meeting of the American Physiological Society stated that the respiratory quotients during rest, work, and recovery were slightly depressed by the ingestion of small amounts of ethyl alcohol. The respiratory quotient of the excess metabolism of work was lower after taking alcohol than in control tests. This suggested to them that the alcohol furnished part of the energy used by the muscles in doing work.

A survey of the preceding abstracts will show that the majority of the investigators believe that their results indicate that alcohol is utilized or burned in muscular work. Those who have come to the conclusion that their work disproves or minimizes the availability of alcohol for muscular activity are in the minority. They are Chauveau (7), Terroine and Bonnet (21), Galamini (9) and Meyer (17). However, none of these can be considered as critical experiments. The dose in Chauveau's experiments was large for normal performance and the work began too soon after the alcohol was taken. The work of Terroine and Bonnet was not a direct attack on the effect of muscular work itself on the metabolism of alcohol, and their results are so at variance with all preceding work on the effect of alcohol on metabolism that they must be considered with a good deal of reserve. Their observations, however, are distinctly challenging, and experiments of this type should be made as their method of study is a logical one. The observations of Galamini were relatively few and the time relationships were so irregular that it is difficult to compare the results under the conditions at rest with those at work. In fact, the few results that the author has given would indicate that muscular work prevented the rise in alcohol in the blood that occurred at rest and thus alcohol was more rapidly burned during work than during rest. Meyer's results on the respiratory quotient after the ingestion of 240 cc. of absolute alcohol are simply astounding and would indicate that there is a possibility of large quantities of alcohol preventing its combustion rather than accelerating it.

On the positive side the studies may be classified according to nitrogen balance and excretion, respiratory exchange, and alcohol in the blood. The studies on the nitrogen excretion may be considered as of the least importance and of the least significance. Nitrogen excretion is bound up with so many factors, such as accuracy of determination of nitrogen intake,

digestibility of food nutrients, diuresis, nitrogen in feces, nitrogen in sensible perspiration, and the maintenance of exactly the same amount of occasional muscular activity other than that due to the prescribed exercise, that it is questionable whether it would be possible to demonstrate by such a study whether muscular activity accelerated the rate of combustion of alcohol. Consequently the reviewer does not believe that the investigations of Krieger (14) and Sommerkamp (20) are decisive.

Of the studies in which the respiratory exchange was used as the criterion, three (v. Hoogenhuyze and Nieuwenhuyse, (22), Brechmann, (2), (3)) were made with the Zuntz-Geppert gas-analysis apparatus and the reviewer does not believe that the Zuntz-Geppert apparatus is in general suitable for such critical work as the differentiation between the amount of combustion of a substance in rest and work. Furthermore, all three of these studies were additionally complicated by the taking of two meals during the course of the measurements. The reviewer does not wish to imply that the results do not indicate that alcohol was burned, for the respiratory quotients in all three studies showed that alcohol depressed the quotient both during rest and either immediately following or during work. But it is impossible to draw the conclusion that muscular work accelerated the combustion of alcohol as the data are insufficient to apportion the metabolism among protein, carbohydrate, fat, and alcohol. The results simply indicate that after alcohol is ingested the combustion was rapid enough both during rest and work to cause a fall in the respiratory quotient. Probably no student of metabolism would deny that alcohol is burned in the animal body, at least to the extent of 90 per cent of the amount ingested. We know of no other means of its disposal at the present time. The investigation of Durig (8) can not be considered as decisive because of the admixture of food with the ingestion of alcohol. The preliminary report of the investigation of Hitchcock and Kraft (13) lacks details, so no critical assessment can be made at this time. When the respiratory exchange is used as a criterion of the influence of muscular activity on the metabolism of alcohol, it is necessary to have a method of measurement which can be considered free from possible objections such as questions of normality of breathing, method of measuring total metabolism, analysis of gases and methods of sampling. The respiratory exchange should be measured over the same period during rest as during work and the measurements in the muscular work experiments should include the recovery period as well as the periods of muscular activity. Control experiments both during rest and activity of the same duration and intensity of work are absolutely necessary to calculate the amount of alcohol burned when the respiratory ex-

change is used as a criterion. Alcohol has an effect on the subject, at rest at least, which tends to obscure the significance of respiratory quotients obtained following its ingestion. A subject tends to become drowsy and very easily drops off to sleep if efforts are not made either on the part of the subject himself or on the part of the observer to keep him awake. It is well known that drowsiness or sleep tends to cause the respiratory quotient to fall and that the breathing is irregular during the transition period from the condition of being alert and awake to the condition of sound sleep. Thus the interpretation of the respiratory quotients obtained under these conditions is attended with difficulties. There is also to be considered the physiological effect of alcohol on the sensitivity of the respiratory center and on the acid-base equilibrium and reaction of the blood. Higgins (11) found that alcohol was either without action on the respiratory center or that it sometimes acted to increase the sensitivity of the respiratory center as indicated by a drop in the carbon dioxide tension of the alveolar air. Himwich and co-workers (12) have reported recently on the effect of alcohol on the lactic acid and CO_2 content of the blood and call attention to the necessity of taking into consideration the rapid shifts of the acid-base equilibrium in the interpretation of respiratory quotients after the ingestion of alcohol. Even when all of these conditions are met, there is still the question of what nutrients alcohol replaces and in what proportions. We do not know whether alcohol diminishes the metabolism of protein, fat, and carbohydrate according to the proportions existing before the alcohol was ingested, or replaces one substance more than the others.

In considering the third criterion of metabolism (combustion) of alcohol, namely, the alcohol content of blood or tissues, it is found that all but one (Miles, 18) show that muscular activity affects the concentration of alcohol in the blood or tissues in such a way that after muscular activity, the concentration of alcohol is lower than when the subject has rested the entire period under observation after the ingestion of alcohol. Miles had only one experiment under each condition and his dose was combined with 300 cc. of unfermented grape juice. One does not know whether the grape juice would prevent the combustion of alcohol in muscular work or not, but these two experiments can not be considered entirely free from possible objection because of the presence of readily oxidizable material in the grape juice. Miles' main problem was not the effect of muscular activity on alcohol concentration in the tissues but the comparison of alcohol concentrations in the blood and urine. Carpenter's (5) study was indirectly indicative and depended so much upon a qualitative assessment of a graphic record of activity that it can not be considered rigorously decisive.

The experiments of Grehant (10) and Mellanby (16) with dogs are both consistent in indicating that muscular exercise promotes the more rapid disappearance (presumably combustion) of alcohol from the tissues. There is an inconsistency in that with the higher amounts in Mellanby's experiments, there was no apparent effect on the alcohol concentration in the blood as the result of exercise. The studies of Galamini (9) and Cassinis and Bracaloni (6) on men also appear to be consistent with the general thesis that exercise accelerates the combustion or disappearance of alcohol in the body. The reviewer is of the opinion that there is a discrepancy between the calculation of the amounts of alcohol metabolized when the respiratory exchange is used as a basis for the computation, and when the alcohol in the tissues is used as a basis. Carpenter (4) called attention to the discrepancy in the time relationships between the metabolism of alcohol as indicated by the respiratory exchange and as indicated by the concentration of alcohol in the urine, and later (5) to the discrepancy between the alcohol that could be accounted for by metabolism and concentration of alcohol in the tissues of hens, and that actually absorbed during the exposure to alcohol vapor. In both groups of experiments the measurements of respiratory exchange and alcohol in urine of men and of absorption and alcohol content of tissues in hens were not made in each case on one and the same day, and therefore lack the unity that experiments of this kind would have if all classes of observations were made in the same experiment. Experiments are needed in which more than one of the criteria are used as a basis of interpretation. The alcohol content of the blood is undoubtedly the best method of determining the rate of disappearance of alcohol when the observation lasts until all alcohol has disappeared from the blood. The determination should be supplemented by the determination of the alcohol in the expired air and in the urine in order to have additional information on the paths of alcohol elimination. In fact, when it is not feasible to obtain samples of blood frequently, the alcohol in expired air can be used as an index of the changes in the blood, for Liljestrand and Linde (15) have shown that the alcohol in the blood and expired air undergo parallel changes at rest, and similarly, Miles (18) has shown that the alcohol in blood and urine are parallel at rest and during work, but not identical in concentration. The amount of alcohol in the expired air should be known because of the greater respiratory ventilation during work than during rest. The quantity of alcohol eliminated in the expired air might be of significance in lowering the alcohol content of the body. The best type of study of the metabolism of alcohol at rest and at work would therefore include the measurements of the respiratory exchange and the alcohol in

the blood, urine, and expired air as frequently as feasible continued until the alcohol has disappeared from the tissues. The reviewer is of the opinion that the literature by no means furnishes adequate data to enable one to draw a decisive conclusion in regard to whether or no muscular activity accelerates the combustion of alcohol. Although some of the previous work on the metabolism of alcohol during muscular work has been under the condition of accompanying ingestion of food, the experiments were not carried out for the purpose of determining the effect of food ingestion on the metabolism. The field of the study of the effect of food ingestion on the metabolism of alcohol during work has not been investigated, consequently there remains much further work to be done on the metabolism of alcohol as affected by muscular activity, either when given alone or in combination with various classes of nutrients.

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